DISCONTINUOUS RESPIRATION IN INSECTS: ROLE OF THE SPIRACLES[1]

HOWARD A. SCHNEIDERMAN

Department of Zoology, Cornell University, Ithaca, New York

In many groups of insects[2] metabolic carbon dioxide is retained within the insect and released during brief periods in "bursts" (Punt, 1944, 1948, 1950a, 1950b, 1956; Schneiderman, 1953; Schneiderman and Williams, 1953–1955; Buck *et al.*, 1953; Buck and Keister, 1955; Ito, 1954). In diapausing pupae of the Cecropia silkworm, for example, more than nine-tenths of the metabolic carbon dioxide is stored and then released in brief bursts, which occur from once every week to many times per hour, depending on the temperature and metabolic rate. The remaining carbon dioxide escapes during the interburst period. When measured by usual manometric procedures, the uptake of oxygen, unlike the release of carbon dioxide, appears continuous and almost steady (Schneiderman and Williams, 1953a, 1955; Buck and Keister, 1954, 1955). If the spiracles are sealed with paraffin, virtually all the respiratory exchange ceases (Ito, 1953; Schneiderman and Williams, 1955); the spiracles are, therefore, the site of both the discontinuous release of carbon dioxide and the simultaneous continuous uptake of oxygen. When metabolic rate is low, the bursts are accentuated. Thus at 10° C. carbon dioxide may be given off only once a week and the interburst rate of carbon dioxide may be but 1/100th the rate of oxygen uptake. This indicates the true dimensions of the respiratory paradox: oxygen enters the spiracles during the interburst period at many times the rate at which carbon dioxide leaves and, furthermore, the insect releases its carbon dioxide periodically.

The central importance of spiracular behavior in the discontinuous release of carbon dioxide was suggested by the observation of Buck and his co-workers (1953, 1955) that intubating the spiracles of *Agapema* pupae eliminated the bursts of carbon dioxide. Subsequently, we observed that excision of the valve from one of the fourteen functional spiracles of a Cecropia pupa caused carbon dioxide output to become continuous (Schneiderman and Beckel, 1954; Schneiderman, 1956). The only maneuver that restored the discontinuous release of carbon dioxide was the sealing of the open spiracle. It appears from this experiment that all the spiracles

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[2] Groups include larval and adult Hemiptera (*Rhodnius prolixus; Triatoma rubrofasciata, Cimex lectularius;* adult Dictyoptera (*Periplancta americana*); larval and adult Orthoptera (*Locusta migratoria*); adult Coleoptera (*Carabus nemoralis, Meloë proscarabaeus, Hadrocarabus problematicus*); diapausing larvae of Lepidoptera (*Arctia* sp.); diapausing pupae of Lepidoptera (*Hyalophora cecropia, Antheraea polyphemus, Samia cynthia, Rothschildia oryzaba, Agapema galbina, Bombyx mori* ("dauer pupae"), *Sphinx ligustri, Agrotis* sp., *Papilio machaon*); non-diapausing pupae of *Bombyx mori*, diapausing adults of Lepidoptera (*Vanessa urticae*).

remain nearly closed during the interburst, preventing carbon dioxide from diffusing out in substantial quantities, and that one or more of the spiracles opens during a burst. The spiracular valves thus hold an important key to the discontinuous release of carbon dioxide.

Several theories have been proposed to explain the disparate rates of oxygen and carbon dioxide transfer during the interburst period, as well as the bursts themselves (Punt, 1944, 1950a; Punt *et al.*, 1957; Zeuthen, 1955; Buck *et al.*, 1953; Buck and Keister, 1954, 1955; Schneiderman, 1956). The most recent and comprehensive theoretical analysis of the entire phenomenon is that of Buck (1958a, 1958b). This theory and all of the others depended for the most part upon (a) indirect estimations of tracheal carbon dioxide and oxygen tensions during the "burst cycle," (b) postulated behavior of the valves that regulate the opening of the spiracles, (c) assumed changes in intratracheal barometric pressure for which there was no empirical evidence whatever, (d) hypothetical changes in the volume of the tracheal system, (e) cataclysmic biochemical changes. To test these theories and to resolve the paradox, it proved necessary: (a) to continuously record the behavior of the spiracular valves during the burst cycle, (b) to measure directly the changing composition of tracheal gases during the cycle, (c) to measure the changing intratracheal barometric pressure, and (d) the changing tracheal volume during the cycle. The present report initiates a series in which methods will be described that accomplish these objectives and provide fairly precise pictures of both the partial and absolute pressure gradients driving oxygen into the insect, the gradients driving carbon dioxide out of the insect, and cyclic variations in the aperture of the spiracles and in the volume of the tracheal system. The results to be reported confirm that the breathing of silkworm pupae involves processes other than physical diffusion, and also bear out many of the theoretical predictions of Buck (1958b). However, in some essential points, they do not support his theory, but instead provide evidence for another kind of insect breathing which seems different from any previously proposed or demonstrated and which may account for discontinuous respiration. In addition, they define the stimuli that cause the cyclical activity of the spiracles.

The first paper focusses on the role of the spiracles in discontinuous respiration. A preliminary account of some of these results has been given elsewhere (Schneiderman and Beckel, 1954; Schneiderman, 1956).

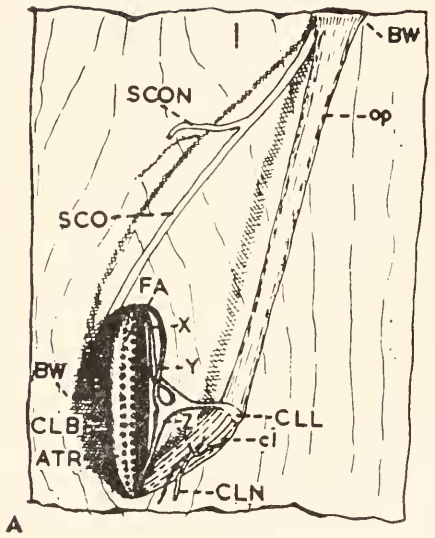
Materials and Methods

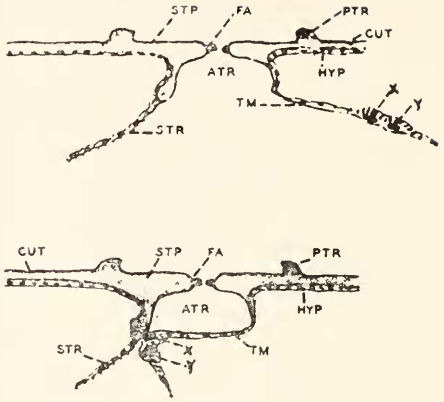
1. *Experimental animals*

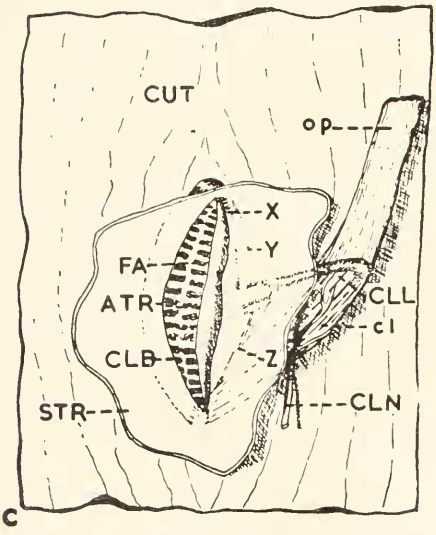
Experiments were performed on diapausing pupae and developing adults of the giant Saturniid silkworms *Hyalophora cecropia, Antheraea polyphemus* and *Samia cynthia*. In our experience these species of closely related moths behave in virtually identical fashion in the sorts of experiments that were undertaken. Animals were reared on net-covered trees or purchased from dealers and stored at 25° C. and 60% to 70% relative humidity. All experiments were conducted at 25° ± 1° C.

2. *Respiratory measurements*

Respiratory exchange was determined manometrically by techniques previously described (Schneiderman and Williams, 1953a, 1955). Measurements were per-







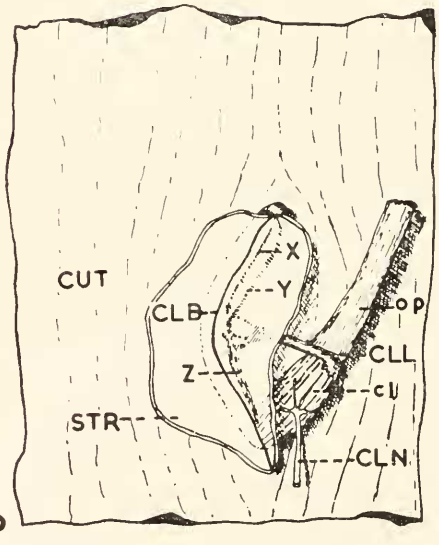


FIGURE 1. (A) Pupal spiracular regulatory apparatus of Cecropia, as seen from inside the animal. No tracheae are shown. *ATR*, atrium; *BW*, body wall; *cl*, closer muscle; *CLB*, closing bow; *CLL*, closing lever; *CLN*, nerve of closer muscle; *FA*, filter apparatus or stigmal plate; *OP*, elastic opener; *SCO*, scolopophorous organ; *SCON*, nerve of scolopophorous organ; *X*, dorsolateral closing bar; *Y*, dorsomedian closing bar; *Z*, ventral closing bar. (B) Frontal sections through the spiracular region, above the closing lever, and cutting the two dorsal closing bars. These show how the spiracle closes when the closing bars are pushed against the closing bow or atrium. *CUT*, cuticle; *HYP*, hypodermis; *PTR*, peritreme; *STP*, stigmal plate, *STR*, spiracular tracheal manifold; *TM*, tracheal membrane. (C) Apparatus viewed from the inside with the scolopophorous organ and part of the elastic opener removed. Only part of the spiracular tracheal manifold is shown. The valve is open. (D) Same as (C) only the valve is closed.

formed by the "direct manometric method" (Umbreit *et al.*, 1958) in 45-cc. cylindrical vessels equipped with venting plugs and adapters, for use with standard Warburg manometers.

3. *Recording of spiracular movement*

(a) *Anatomy of the spiracle*

These silkworm pupae have a pair of thoracic spiracles and six pairs of functional abdominal spiracles. Each spiracle is surrounded by a chitinous peritreme, and covered by a stigmal plate, or filter apparatus, which communicates by a thin slit to a chamber below—the atrium—which contains the spiracular valve. Gas exchange between the atrial chamber (and hence the atmosphere) and the interconnecting tracheal system, which lies just below it, is regulated by this valve. The morphology of the spiracular apparatus and some of the physiological properties of the spiracular muscle of pupae of Saturniid moths have been described most recently by Beckel (Beckel, 1955, 1958; Beckel and Schneiderman, 1956, 1957). For our present purposes, it is sufficient to note that the spiracular valve is an epithelial membrane which is firmly attached to a chitinous frame consisting of a bow and three bars which unite in the middle to give rise to a lever (Fig. 1). A closer-muscle stretches from the ventral tip of the lever to the ventral corner of the valve. It is opposed by an elastic ligament which extends from the dorsal tip of the lever to the body wall. When the closer muscle contracts, it pulls on the lever and closes the valve. When it relaxes, the valve opens because of the elasticity of the chitinous frame and the tension of the opposing elastic ligament. The muscle is innervated by a nerve from the corresponding segmental ganglion and by a branch of the median nerve of the next anterior segment.

(b) *Recording valve movements*

To expose the valves in the living insect it was necessary to remove the overlying chitinous stigmal plate. This could easily be done under the dissecting microscope after the animal had been anaesthetized with carbon dioxide. Insofar as could be judged, this operation in no way interfered with the normal functioning of the valves, which were now clearly visible. When the valves were so exposed for a week or more, they occasionally dried out and ceased functioning normally. To prevent this, after each period of observation, the spiracular opening was sealed with Tackiwax.[3] Because of their greater accessibility, the abdominal spiracles were examined in preference to the thoracic pair. Of these the first, second, and third abdominal were the easiest to study because they served non-collapsible segments and were not obscured when the animal moved its abdomen.

Several methods for recording spiracular movements of intact insects have been employed in the past and there have been several descriptions of spiracular behavior in the cockroach *Periplaneta americana* (Hazelhoff, 1926a, 1926b), the phasmid *Dixippus morosus* (Stahn, 1929); the rat flea *Xenopsylla cheopis* (Wigglesworth, 1935; Herford, 1938), the bedbug *Cimex lectularius* (Wigglesworth, 1941), the

[3] After the present experiments were completed, we discovered that a valve can be prevented from drying out by sealing a small transparent plastic window over the exposed spiracle. Although the window prevents gas exchange through the spiracle, it allows convenient observation of spiracular movements, and the valve functions for many weeks.

grasshoppers *Dissosteira carolina* (McCutcheon, 1940), *Schistocerca obscura* (Watts, 1951), and *Schistocerca gregaria* (Hoyle, 1959), the larva of the commercial silkworm (Shimizu and Ono, 1942), the flies *Musca domestica* and *Calliphoga macellaria* (Case, 1956a) and the cockroaches *P. americana* and *Blaberus craniifer* (Case, 1957b). The older literature has been summarized by Hazelhoff (1926a). The present experiments employed a modification of the ocular micrometer system of McCutcheon. A pupa with one or more of its spiracles exposed to view was placed beneath a binocular dissecting microscope furnished with an ocular micrometer and examined under 60 ×. The hairline of the micrometer was focussed on the leading edge of the spiracular valve and was adjusted to follow the valve as it opened and closed (Fig. 2). The crosshairs on the micrometer eyepiece indicated the position of the hairline when the valve was closed. The hairline was moved by a rotating knob whose action was translated into a kymograph trace by means of a system of pulleys and levers and an ink-writing pen recording on a

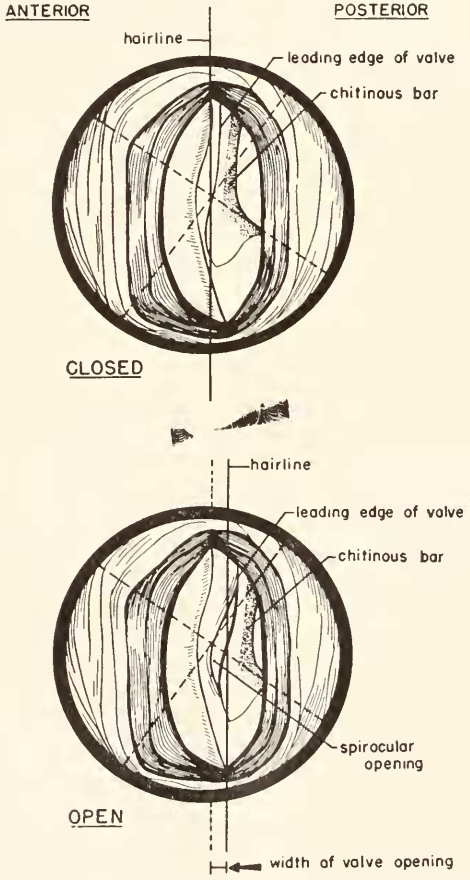


FIGURE 2. Spiracle as seen through ocular micrometer. The filter apparatus and most of the stigmal plate have been removed and the valve is visible. For further details see text.

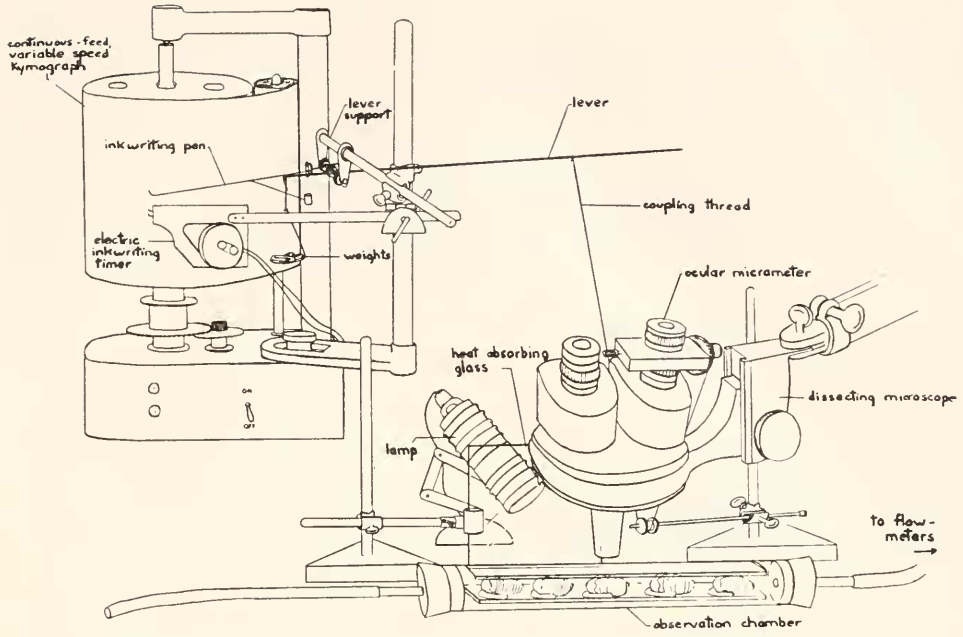


FIGURE 3. Apparatus used for recording spiracular valve movements. The observation chamber shown is of the sort also employed in studies with intubated pupae. For further details see text.

continuous feed kymograph (Fig. 3). The tracings of the ink-writing pen provided a record of the movements of the spiracular valve, each deflection on the record representing a valve movement. The ordinate of the trace shows the width of valve opening in arbitrary units, while the abscissa denotes the duration of the opening. Directly beneath the record of valve movements the time was recorded by an ink-writing timer.

In most of the experiments spiracular behavior was viewed through a 20-cc. glass chamber in which the insect was held secure in a plasticene support, or in a 250-cc. lucite chamber with a flat surface for optical convenience. Air and gas mixtures were flushed through the chamber as desired. The rate of gas flow varied from 25 to about 500 cc./min. in different experiments and appeared to have no effect on the phenomena under investigation. The time required to change the atmosphere in the small chamber was rarely longer than 10 seconds, but for the large chamber it took about two minutes.

Gas mixtures were either pre-mixed in pressure cylinders or made up by proportional flow. All mixtures were analyzed periodically.

A. Experiments With Intact Pupae

1. *Normal behavior of spiracles*

Figure 4 is a portion of a typical record of the activity of the third abdominal spiracle of a Cecropia pupa over a period of four hours. A consistent pattern of

valve movement occurred in cycles lasting 45 minutes to 2 hours. After a period of about 10 minutes, during which it remained closed and motionless, the valve fluttered for between 15 and 40 minutes and then, within a minute, the valve movements progressively increased in amplitude until the valve opened fully. Swaying slightly, it remained open for several minutes. Then it alternately opened and closed for several minutes, and the valve movements gradually decreased in amplitude and duration, until the valve closed altogether. Following this, the valve remained motionless until the fluttering preceding the next period of wide openings. This cycle was repeated over and over, and apparently represented the normal behavior of the spiracle.

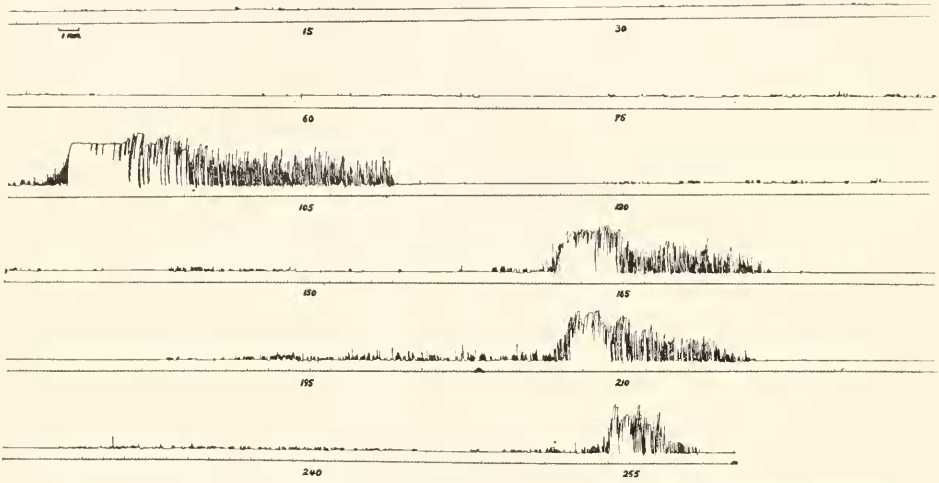


FIGURE 4. Record of the valve movements of a third abdominal spiracle of a diapausing Cecropia pupa over a 4-hour period in air at 25° C. Each mark above the baseline represents a valve opening. Occasional marks below the baseline are artifacts caused by vibration of the apparatus.

Manometric observations of the carbon dioxide output of this pupa revealed bursts of carbon dioxide at intervals of 30 minutes to two hours. A similar correlation between the frequency of carbon dioxide bursts and periods when the valve was widely open has been consistently observed in all other silkworm pupae studied. Moreover, by utilizing techniques which permit simultaneous recording of both valve movements and gas exchange, it has been possible to show the correlation directly (Schechter and Schneiderman, unpublished observations). Hence it seems evident that the periodic bursts of carbon dioxide result from the periodical prolonged openings of the spiracles, which we have termed "spiracular bursts." The term "burst" seems appropriate for both the spiracular and the manometric events, since they coincide. The spiracular burst can conveniently be partitioned into a period of wide openings—the "open phase"—which is followed by a period of rapid closures—the "decline phase." The end of the decline period and of the spiracular burst is marked by the moment the valve constricts tightly. The "interburst"

consists of a "constriction period" after the burst, when the valve appears closed and motionless, and a "flutter period" prior to the next burst.

The flutter period was usually quite irregular in terms of both the frequency and amplitude of the valve movements. As Figure 4 reveals, the flutters usually

TABLE I

Duration of various phases of spiracular burst cycles in a typical series of diapausing Cecropia pupae and developing adults of Polyphemus

Experiment No.***	Cycle length (minutes)	Duration of various phases (minutes)*			
		Interburst		Spiracular burst	
		Constriction	Flutter	Open	Decline
Cecropia					
827	114	13 (11)	91.5 (80)	5.5 (4.8)	4.0 (3.5)
8316	74	10 (14)	61 (81)	1.0 (1.4)	2.0 (2.7)
938	73	26 (36)	42 (57)	1.5 (2.1)	3.5 (4.8)
739	68	26 (38)	37.5 (55)	1.0 (1.5)	3.5 (5.1)
8315	65	12 (19)	49 (75)	1.0 (1.6)	3.0 (4.7)
625 a	63	10 (16)	43 (68)	3.0 (4.8)	7.0 (11.1)
8314	50	16 (32)	29.5 (59)	1.5 (3.0)	3.0 (6.0)
625 b	44	16 (36)	19 (43)	2.5 (5.7)	6.5 (14.8)
725 c	43	12 (28)	26 (61)	2.0 (4.7)	3.0 (6.9)
8311 b	39	12.5 (32)	23.5 (60)	1.0 (2.6)	2.0 (5.1)
439	38	17 (45)	16 (42)	1.0 (2.6)	4.0 (10.5)
8311 c	32	18 (56)	12.5 (39)	1.0 (3.1)	0.5 (1.6)
8311 a	29.5	7.5 (25)	19 (65)	1.5 (5.1)	1.5 (5.1)
Average**	56.35 ± 23.25	15.08 ± 5.71	36.12 ± 21.96	1.81 ± 1.28	3.35 ± 1.82
Average %	100	29.85 ± 12.94	60.38 ± 13.67	3.31 ± 1.52	6.30 ± 3.71
Polyphemus					
522 a	16.5	0	15.0 (91)	—	—
522 b	15.4	0	12.3 (80)	—	—
527 a	10.7	0	6.4 (60)	—	—
527 c	9.7	0	7.0 (72)	—	—
527 d	9.1	0	6.2 (68)	—	—
527 f	8.1	0	5.5 (68)	—	—
5218	7.0	0	4.6 (66)	—	—
113 a	6.0	0	3.5 (58)	1.5 (25)	1.0 (17)
113 b	6.0	0	3.0 (50)	2.0 (33)	1.0 (17)
114	6.0	0	3.6 (60)	1.7 (28)	0.7 (12)
115	5.5	0	2.5 (45)	2.3 (42)	0.7 (13)
521 c	5.5	0	3.5 (64)	—	—
117	4.3	0	2.8 (65)	1.0 (23)	0.5 (12)
521 c	2.7	0	1.4 (52)	—	—
Average	8.04 ± 3.98	0	5.52 ± 3.84	1.70	0.78
Average %	100	0	64.21 ± 11.94	30.2	14.2

* Figures in parentheses record the average per cent of the cycle length.

** ± standard deviation.

*** In this and subsequent experiments, the first two digits refer to the particular animal employed, *i.e.*, all experiments beginning with "82" refer to pupa No. 82.

came in volleys which lasted from 10 seconds to 8 minutes and which were punctuated by closed periods which lasted from 20 seconds to several minutes. Less commonly, flutters occurred singly. Frequency varied from short volleys of about one per second to long volleys of one opening every two to ten seconds. The openings were invariably brief, commonly lasting less than a second. Amplitude also varied somewhat and an occasional wide opening punctuated a series of smaller ones. There appeared to be no systematic variation in amplitude, however, until a minute or two before the burst.

Several hundred hours of records of such cycles have been obtained from about 30 individual pupae. Table I records the duration of the various phases of several typical cycles recorded from diapausing Cecropia pupae and developing adults of Polyphemus. Although the cycles that were studied varied in frequency from 20 per hour to one every two hours, the pattern of valve movements in all cases was remarkably similar. The only variation occurred in pupae with cycles of 15 minutes or less. Here the constriction period was lacking and the spiracular valve was in constant slight motion (see Figures 6 and 7). Also, in pupae with brief cycles the frequency of flutters was considerably greater than in pupae with long cycles, and the spiracular bursts occupied a larger and larger proportion of the cycle.

Simultaneous observations of two or three spiracles on the same side indicated that the spiracles were coordinated. When the valves opened in a burst or closed at the end of a burst, they did so within a minute of each other, though when they fluttered, the pulsations were not in exact synchrony. For our present purposes, these observations indicate that recording the behavior of one spiracle provides an accurate picture of the behavior of all the spiracles of the animal, except possibly the thoracic spiracles whose behavior we have never succeeded in observing.

Previous experiments (Punt, 1950a; Schneiderman and Williams, 1955; Buck and Keister, 1955) pointed out that various factors such as metabolic rate, oxygen and carbon dioxide tensions, etc., profoundly affected the cyclical release of carbon

TABLE II

Burst cycle of a Polyphemus during adult development

Days of adult development	Cycle length (minutes)	Spiracular burst duration (minutes)	Amount of interburst fluttering*
−13	120.0	6.8	+
0	9.8	3.3	++
2	7.0	2.4	++
3	5.0	2.7	++
4	5.4	2.0	++
5	4.4	1.7	++
6	4.7	2.0	+++
9	4.0	1.7	+++
10	4.3	2.0	+++
15	2.7	1.3	++++
16	0	0	++++
20 (emergence)	0	0	++++

* +: Normal amplitude, moderate frequency; ++: normal amplitude, high frequency; +++: greater than normal amplitude, high frequency; ++++: fluttering about half-open position, high frequency.

dioxide. Recognizing the key role of the spiracular valves, it was reasonable to anticipate that these several factors would influence the movement of the spiracular valves. This is clearly shown in the following experiments.

2. *Effects of metabolic rate on the behavior of the spiracular valves*

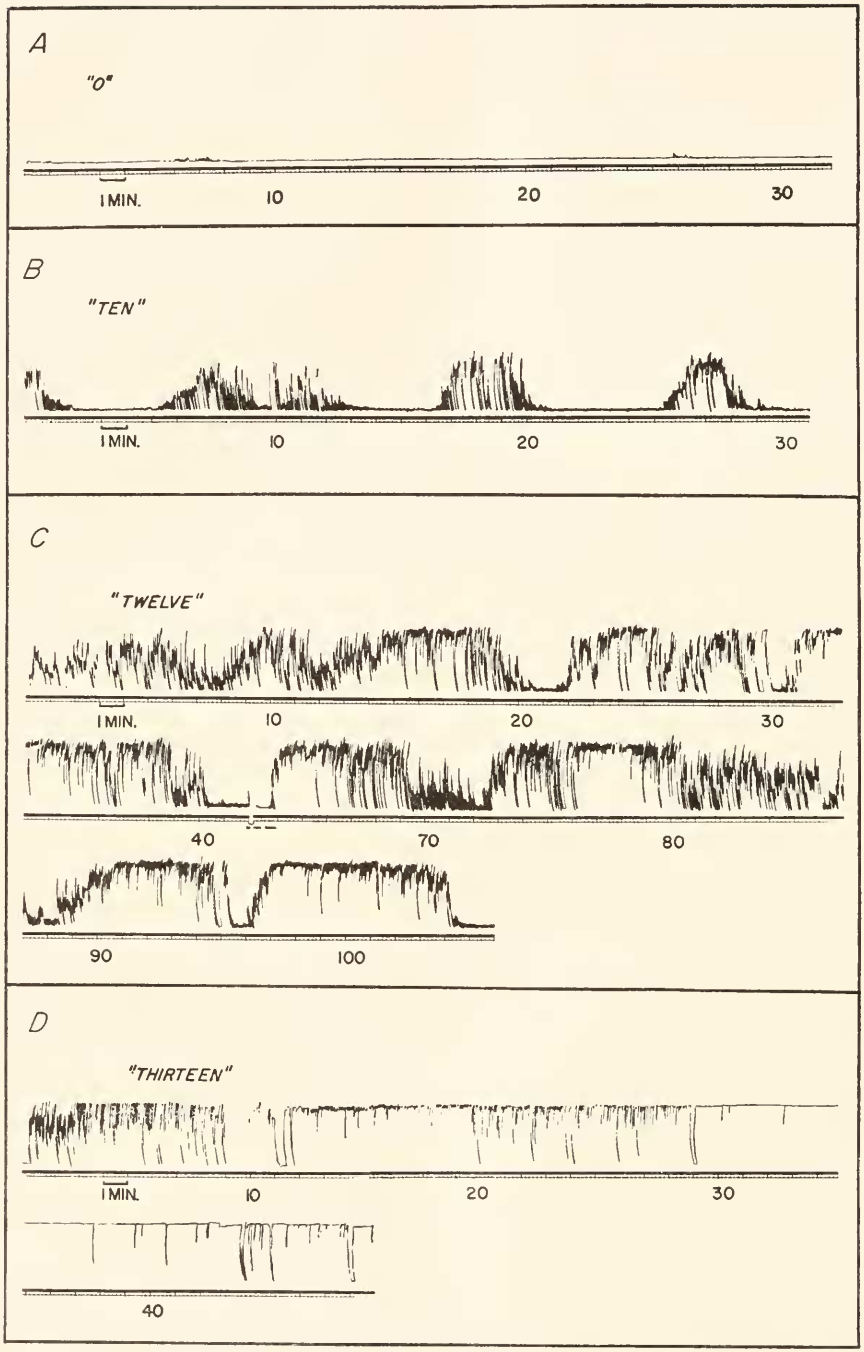
During the pupal-adult transformation, oxygen uptake rises markedly, the cycles of carbon dioxide release become more frequent and the interburst rate of carbon dioxide output increases until the cycles disappear and carbon dioxide is released continuously (Punt, 1950a; Schneiderman and Williams, 1955). Table II summarizes the spiracular behavior of a Polyphemus during adult development, and reveals that the spiracular bursts increased in frequency and that the amount of interburst fluttering also increased, first in frequency and then in amplitude, until eventually on the sixteenth day of the 21-day period of adult development, the spiracular bursts disappeared and the spiracles fluttered continuously about a half-open position. It is important to note that in insects with very high metabolic rates the flutters had far greater amplitude than normal interburst flutters and resembled the spiracular movements encountered in the decline period after a spiracular burst. In any case, by using developing adults, it was possible to obtain animals with brief burst cycles. Another useful means of securing animals with brief cycles for convenient study was to injure pupae. Injury provokes a prompt increase in metabolic rate, which persists for days, and a tremendous increase in burst frequency (Schneiderman and Williams, 1955). In a typical experiment excision of the facial region from a Polyphemus pupa increased cycle frequency from one spiracular burst every two hours before injury, to 12 per hour four days after injury.

The explanation for these effects of metabolic rate on the spiracular burst cycle seems clear. One of the probable consequences of increased metabolic rate is to lower tracheal P_{O_2} and increase tracheal P_{CO_2}. It seems likely that these factors are directly responsible for the acceleration of the burst cycle. This is confirmed in the following experiments on spiracular occlusion.

3. *Effects of spiracular occlusion*

In pupal silkworms, the spiracles are the sole gateways to the tracheal system and their occlusion affords a simple method of lowering internal P_{O_2} and raising internal P_{CO_2}. The effects of spiracular occlusion on the cycle of valve activity of a Polyphemus pupa are seen in Figure 5. Sealing five pairs of abdominal spiracles with paraffin called forth an immediate increase in spiracular burst frequency from about one burst per hour to 6 or 8 per hour. After 12 or 13 of the 14 spiracles were sealed, the spiracular bursts disappeared completely. Unsealing the spiracles initiated the return to substantially normal behavior.

In pupae with five pairs of abdominal spiracles sealed and a spiracular burst frequency of 5 or 10 per hour, it was occasionally possible to restore normal burst frequencies of one to two per hour by exposing the pupa to pure oxygen. The increased oxygen also decreased interburst fluttering and led to wider openings at the time of the spiracular burst. The implications of these observations will be examined in the Discussion. For our present purposes suffice it to say that these spiracular occlusion experiments indicate that low oxygen combined with high carbon dioxide increases spiracular valve movements and speeds up the burst cycle.


A
"0"
1 MIN.
10
20
30
B
"TEN"
1 MIN.
10
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30
C
"TWELVE"
1 MIN.
10
20
30
40
70
80
90
100
D
"THIRTEEN"
1 MIN.
10
20
30
40

FIGURE 5.

TABLE III

Effects of P_{O_2} on the various phases of the spiracular burst cycle of two diapausing Cecropia pupae (S-82 and S-93)

Expt. No.	Ambient P_{O_2}	Average cycle length (minutes)	Mean duration of various phases (minutes) Interburst*				Spiracular burst	
			Constriction	Percentage change in constriction period**	Flutter	Percentage change in flutter period**	Open	Decline
827	21	114	13 (11)	+462	91.5 (80)	−100	5.5 (4.8)	4.0 (3.5)
827a	100	75	73 (97)		0 (0)		2.1 (2.8)	0 (0)
938a	5	Continuous fluttering	—	—	—	—	—	—
939a	15	73	11.5 (16)	−36	58 (79)	+32	1.4 (1.9)	1.5 (2.1)
930 (Av.)	21	66	18.0 (28)	0	44 (65)	0	1.2 (1.9)	3.0 (4.7)
9310	35	50	28 (56)	+56	18 (36)	−59	2.0 (4.0)	2.0 (4.0)
9311	75	61	58 (95)	+220	0 (0)	−100	1.0 (1.6)	2.0 (3.3)

* Figures in parentheses record the *average percentage of the cycle length* occupied by the particular phase. Most of the results represent the average of two cycles except the results in air (930) which represent the average of five cycles of 50 to 74 minutes duration.

** Compared with air controls.

To separate the action of these gases on spiracular behavior, we tested the effects of specific gas mixtures on valve activity.

4. *Effects of oxygen tension on the spiracles*

The effects of decreased oxygen tension on spiracular behavior are evident in Figure 6. This pupa had a high metabolic rate and a brief burst cycle. As Figure 6A shows, within six minutes of lowering the ambient oxygen tension to *15%*, interburst fluttering increased so much that only a semblance of a burst cycle remained. The cycle returned to normal after about five minutes.

As Figure 6B shows, *7%* oxygen completely obliterated the burst cycle. Within one minute the valve opened widely without fluttering, remained open for five minutes with only an occasional closure, and then closed partially five to ten times each minute. Manifestly, this gradual return of spiracular fluttering looks like adaptation, but in our opinion it is more likely the result of a lowered P_{CO_2} within the insect as a result of prolonged spiracular opening (*cf.* Discussion). Within one minute of return to air the valve began to flutter closed and soon thereafter the burst cycle reappeared.

FIGURE 5. Effects of spiracular occlusion on the behavior of the second right abdominal spiracle of a Polyphemus pupa. Air at 25° C. (A) *No* spiracles sealed; 14 functional. A burst occurred just before the recording was begun. For 63 minutes thereafter no bursts occurred. (B) *Ten* spiracles sealed: first, third, fourth, fifth and sixth pairs of abdominal spiracles; four functional. Burst frequency increased from less than one per hour to one every 8 or 10 minutes. The pattern persisted essentially unchanged for 20 hours. (C) *Twelve* spiracles sealed: all of above plus both thoracic spiracles; two functional. Fluttering of valve in open position for periods of 8 to 20 minutes punctuated by brief one-half- to two-minute periods of normal fluttering about the closed position. (D) *Thirteen* spiracles sealed: all of above plus left second abdominal spiracle; one functional. Only the exposed spiracle was available for gas exchange. The result was intermittent closures from a wide-open position, first rapidly and then more slowly.

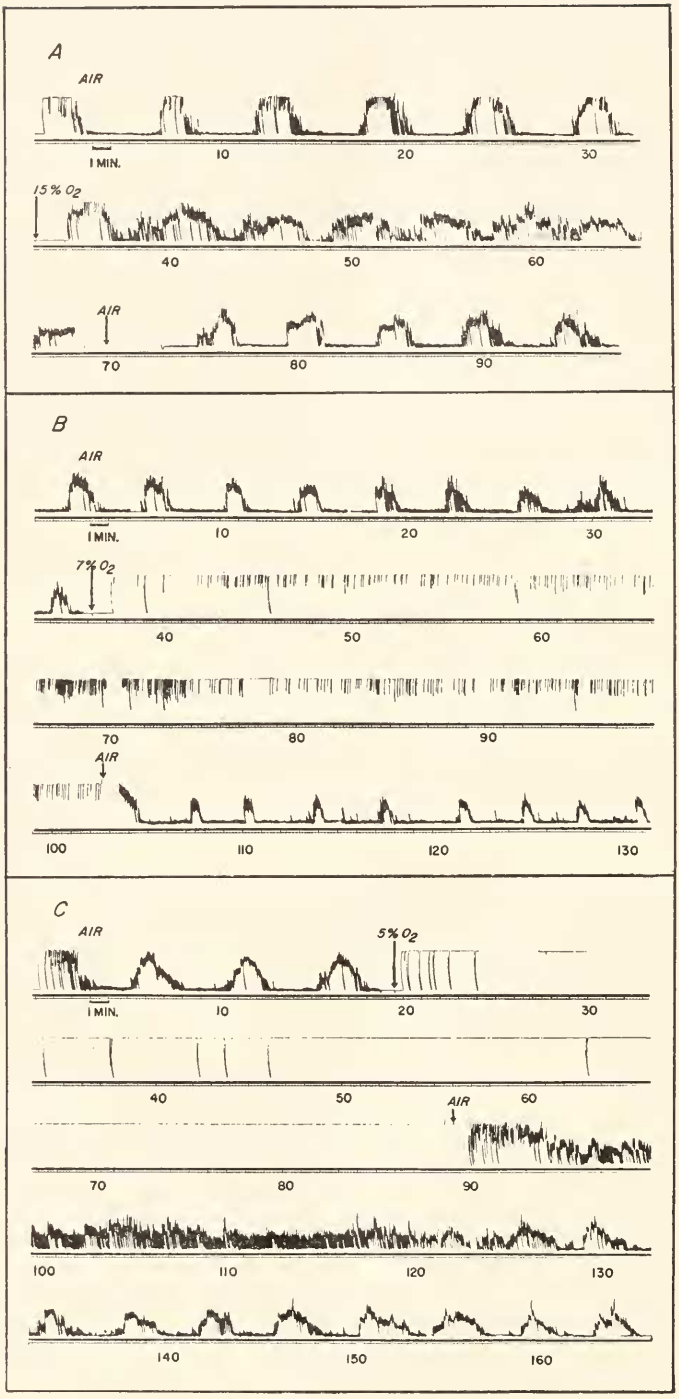
A
AIR
1 MIN.
10
20
30
15% O2
40
50
60
AIR
70
80
90
B
AIR
1 MIN.
10
20
30
7% O2
40
50
60
70
80
90
AIR
100
110
120
130
C
AIR
5% O2
1 MIN.
10
20
30
40
50
AIR
60
70
80
90
100
110
120
130
140
150
160

FIGURE 6.

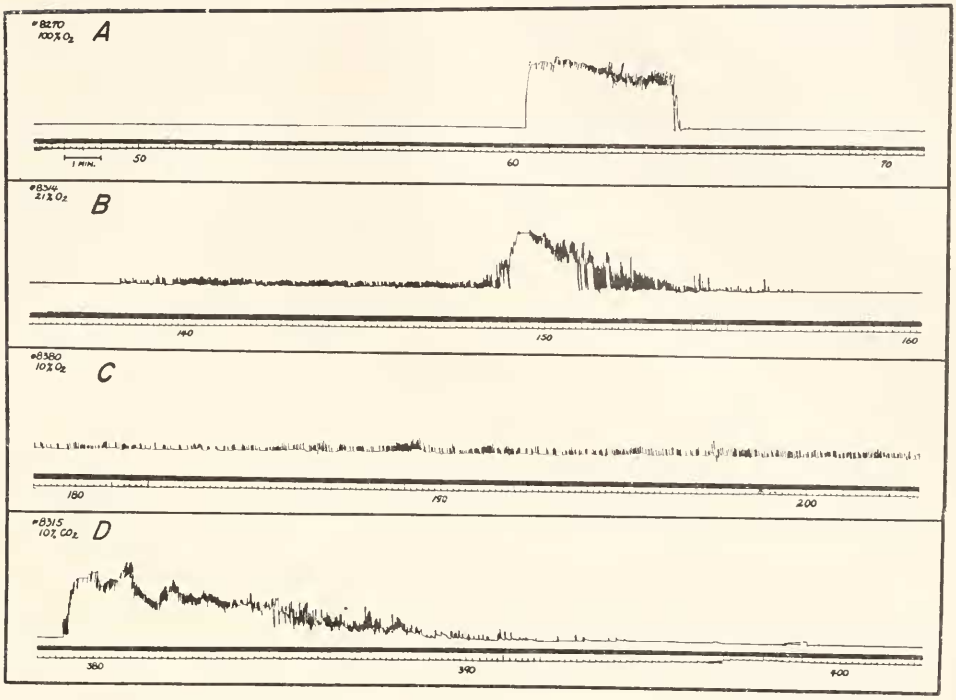

FIGURE 7. Records of spiracular movements of a Cecropia pupa in various ambient gas tensions. In all cases records were made when spiracular activity had reached a steady state. (A) 100% O_2; (B) air; (C) 10% O_2; (D) 10% CO_2. For details see text.

Figure 6C reveals that within 30 seconds of exposure to 5% oxygen, the valve opened widely with no initial fluttering. It gaped open for 70 minutes, closing at irregular intervals for about 40 minutes, and finally stayed wide open and motionless. Within one minute of return to air, fluttering began and continued for 40 minutes, whereupon the burst cycle reasserted itself. Recovery from 5% oxygen thus took nearly forty times longer than recovery from 7% oxygen, suggesting that this low oxygen tension was inadequate for respiration and caused the pupa to become anoxic and build up an oxygen debt. In *pure nitrogen*, a record similar to the 5% oxygen record was obtained (not illustrated). The spiracle closed occa-

FIGURE 6. Effects of P_{O_2} on the behavior of the second right abdominal spiracle of a diapausing Polyphemus pupa. Three records were obtained in studying each gas mixture: in air, in the specfic gas mixture, and again in air, to appraise the recovery of the spiracle. The time required to change the atmosphere in the chamber was less than 15 seconds. In this experiment and in succeeding ones, the reaction time of the spiracle depended very much on the phase of the burst cycle in which the gas was administered. As one would expect, the most rapid responses occurred when gases were admitted during a spiracular burst. In experiments illustrated the gases were added midway in the interburst period. (A) *15% O_2*. Record of valve movements in air, in *15% O_2 + 85% N_2*, and in air. (B) *7% O_2*. Record of valve movements in air, in *7% O_2 + 93% N_2*, and in air. (C) *5% O_2*. Record of valve movements in air, in *5% O_2 + 93% N_2*, and in air.

sionally for about 10 minutes, but then gaped widely, closing only once during thirty minutes of observation. From these results it appears that decreased oxygen tension can cause valve opening.

A clearer picture of the effects of oxygen tension is seen in Table III, which records the effects of several oxygen tensions on the spiracular behavior of two pupae with longer burst cycles than the pupa just described. These pupae took somewhat longer to respond to both high and low oxygen tensions, often more than 10 minutes except when the gas was administered during a spiracular burst. Figure 7 illustrates portions of several representative records. In *5% to 10% oxygen* the cycles were absent and the valves were continuously in motion. The openings were larger than normal interburst openings, but smaller than normal burst openings. The frequency of flutters, however, was not very different from typical interburst flutters and remained irregular. The period between volleys, however, was much decreased and rarely exceeded 20 seconds, whereas in air, two-minute intervals between volleys of flutters were common.

In *15% oxygen* the bursts reappeared. As the oxygen concentration increased above 15%, the width of valve openings in the interburst decreased, while the frequency remained essentially the same. Also, as oxygen tension increased, the fluttering period got progressively shorter and the constriction period progressively longer. Finally, in pure oxygen all the fluttering before and after the spiracular burst was completely eliminated: the valve remained closed until it abruptly shot open in the burst, and then almost as abruptly closed until the next burst. During the spiracular burst itself, at oxygen tensions above 15%, the higher the oxygen tension, the wider was the valve opening. From these results it appears that oxygen tension affects primarily interburst fluttering but also has some minor effects on the burst itself. It is also noteworthy that increasing or decreasing oxygen tension failed to have any predictable effect on burst frequencies: sometimes frequencies were lowered, other times increased.

TABLE IV

*Effects of P_{CO_2} on various phases of the spiracular burst cycle of a diapausing Cecropia pupa (No. S-83)**

Expt. No.	Ambient P_{CO_2}	Average cycle length (minutes)	Average duration of various phases (minutes)**					Percentage change in spiracular burst	Open	Decline
			Constriction	Percentage change in constriction period	Flutter	Percentage change in flutter period	Spiracular burst			
8311	Air	34	10 (29)	+30	21 (62)	−5	3.1 (9.2)	+62	1.4 (4.1)	1.7 (5.1)
	5%	38	13 (36)		20 (50)		5.0 (13.7)		1.8 (5.1)	3.2 (8.6)
8314	Air	50	16 (32)	+13	29.5 (59)	−81	4.5 (9.0)	+67	1.5 (3.0)	3.0 (6.0)
	8%	31	18 (59)		5.5 (18)		7.5 (24.0)		3.0 (9.7)	4.5 (14.3)
8315	Air	65	12 (19)	0	49 (75)	−91	4 (6.2)	+150	1.0 (1.6)	3.0 (4.7)
	10%	26	12 (45)		4.5 (17)		10 (38)		4.0 (15.0)	6.0 (22.5)
8316	Air	74	10 (14)	—	61 (81)	—	3 (4.1)	—	1.0 (1.4)	2.0 (2.7)
	15%	Continuous flutter	—		—		—		—	—

* Experiments were performed on successive days and the normal and experimental records for each day are paired. Most of the results represent the average of two cycles.

** Figures in parentheses record the *average percentage of the cycle length* occupied by the particular phase.

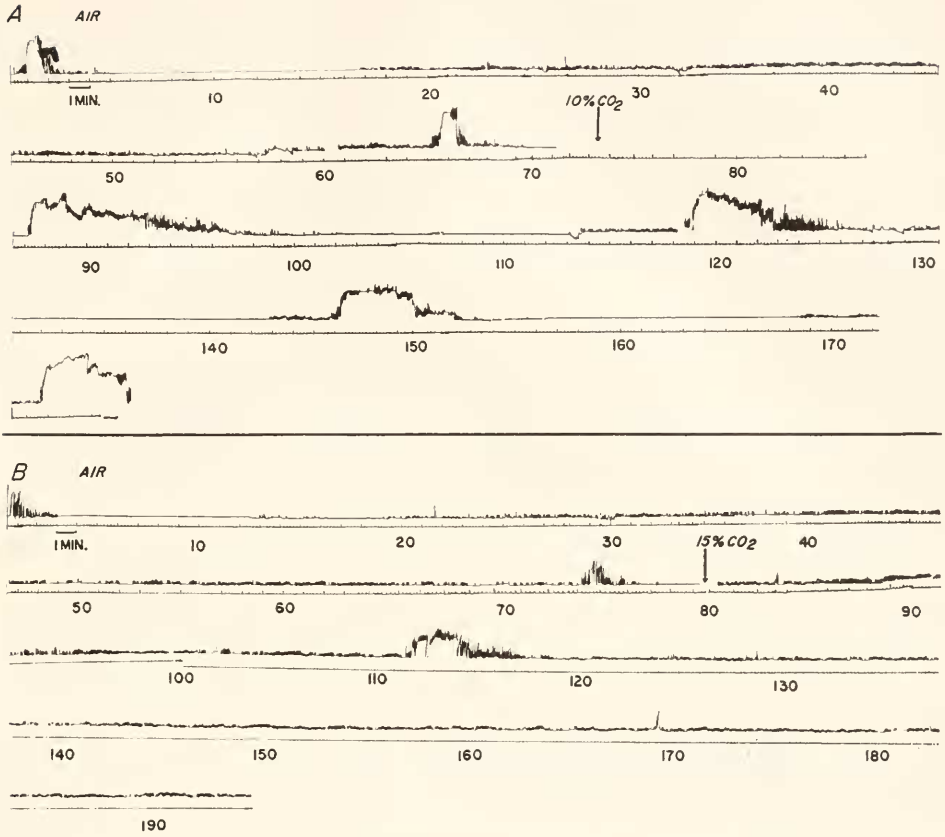

FIGURE 8. Records of spiracular movements of a Cecropia pupa with a moderately long burst cycle in air, followed by 10% or 15% CO_2. (A) 10% CO_2; (B) 15% CO_2.

In occasional experiments, particularly with pupae with high metabolic rates and frequent bursts, pure oxygen failed to suppress interburst fluttering. Instead the spiracular bursts disappeared and the spiracles fluttered continuously. Possible reasons for this curious behavior will be offered in the Discussion.

5. *Effects of carbon dioxide tension*

Increasing the P_{CO_2} increased burst frequency and lengthened both "open" and "decline" phases of the cycle (Table IV and Fig. 8). Besides the effect on burst frequency, the table and figure reveal that high tensions of carbon dioxide also lengthened both the open phase of the spiracular burst and the decline phase after the burst. Thus, in 10% carbon dioxide, the open phases were nearly three times as long as in air (compare Figure 7D with 7B). In addition, although increasing P_{CO_2} had little effect on the duration of the constriction period, it greatly shortened the flutter period until, in 10% carbon dioxide, fluttering was reduced to only a few minutes (Fig. 8). Between 10 and 15% carbon dioxide the burst cycle broke down completely, so that in 15% carbon dioxide the valves fluttered continuously (Fig. 8),

and after an initial spiracular burst, no further bursts occurred. The openings were a bit larger than normal interburst flutterings, but much smaller than normal openings in a burst.

The reaction times for a carbon dioxide response never appeared as long as the reaction times for a response to oxygen. Also, pupae with high metabolic rates, and hence frequent bursts, appeared far more sensitive to carbon dioxide than pupae with low metabolic rates. Thus, in a pupa which had a 4- to 5-minute burst cycle, as little as 2% carbon dioxide had a detectable effect on spiracular behavior (prolonged the burst), while 3% eliminated spiracular bursts and provoked continuous wide flutters. Furthermore, when the carbon dioxide tension was raised to 15% in a pupa with such a brief cycle, the valves opened widely, and nearly a half hour

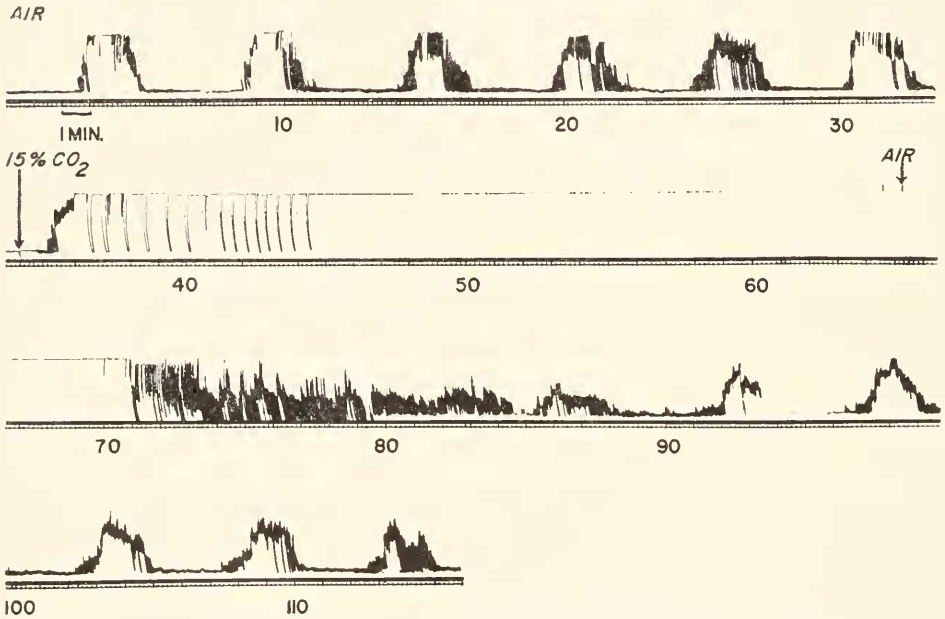

FIGURE 9. Records of spiracular movements of a Polyphemus pupa with frequent bursts in air, in 15% CO_2 + 21% O_2, and in air.

was required for recovery upon return to air (Fig. 9). Such sensitivity to carbon dioxide contrasts markedly with the more modest responses recorded in Figure 8, shown by a pupa with a longer burst cycle. A further observation important to our analysis is that in a pupa in which 3% carbon dioxide eliminated the spiracular bursts, bursts could be restored in 3% CO_2 by raising the oxygen tension to about 90%. This observation suggests that high oxygen tensions decrease the sensitivity of the spiracle to carbon dioxide, a fact to which we shall return.

Although these results convey a general picture of the effects of carbon dioxide and oxygen on the pupal spiracles, they suffer from a conspicuous defect: they provide only little information about the actual gas concentrations *within* the tracheal system that produce a particular effect. In other words, when the insect

is exposed to 15% oxygen or 10% carbon dioxide what are the actual intratracheal oxygen and carbon dioxide tensions? A partial solution to this problem was provided by experiments with intubated pupae which are described below.

B. Experiments With Intubated Pupae

1. *Effects of spiracular intubation*

Just as spiracular occlusion decreases the P_{O_2} and increases the P_{CO_2} within the pupal tracheal system, so spiracular intubation has the opposite effect. In the following experiment, the spiracular valves were exposed in four or five abdominal spiracles on one side of a series of diapausing Cecropia pupae. Into two, three or four of these spiracles short fire-polished glass tubes, about 1 mm. in diameter, were inserted beyond the spiracular valves, placing the tracheal system in free communion with the air. This maneuver caused the P_{CO_2} within the insect to fall and the P_{O_2} to rise, as the gaseous composition of the tracheal system approached that of the outside.

One or two days after the operation, and periodically thereafter for about a week, the unintubated spiracles were examined (usually the second and third abdominal). In every case the spiracular valves appeared to stay tightly constricted for many days. They could be opened, however, by exposing the insect to low oxygen or high carbon dioxide. Thus, although the untouched spiracles of the intubated pupa could still function, their cyclical activity had disappeared, presumably because the normal triggering stimuli for spiracular activity were absent.

When intubations were performed on animals with very high metabolic rates (*e.g.* developing adults), despite four intubated spiracles some spiracular fluttering occurred. Apparently, intubating even four spiracles in animals with high metabolic rates does not permit equilibration of internal and external gases.

These experiments, coupled with the other experiments considered thus far, are consistent with the view that the triggering stimulus for spiracular opening is either low internal tension of oxygen, high internal tension of carbon dioxide, or a combination of the two. This matter is considered in further detail below.

2. *Effects of oxygen tension on the spiracles of intubated animals*

Intubation eliminates the spiracular burst cycle and the spiracles exhibit a persistent pattern of behavior in various gas mixtures. Thus, the intubated pupae provide a simple means of determining the approximate concentrations of oxygen and carbon dioxide that produce a particular kind of spiracular behavior, *e.g.* valve fluttering, bursts etc., and also a means of examining the interaction between carbon dioxide and oxygen in controlling spiracular movement. To conduct these experiments, pupae with two or three intubated spiracles were exposed to various gas mixtures in a flow system, and the movements of two normal spiracles observed until they exhibited a constant behavior. It took anywhere from 5 to 35 minutes for the constant response to assert itself, except in very low oxygen tensions or very high carbon dioxide tensions where the response appeared more rapidly. The decision to study the final response of the spiracles rather than their initial response was taken for several reasons. The principal one was that, in these large insects, it very likely takes many minutes for gases to reach a steady-state. A second

TABLE V

*Determination of the "O_2-open" threshold of two spiracles of an intubated Cynthia pupa**

Per cent oxygen	Response of spiracle 2R	Response of spiracle 3R
21	Closed	Closed
15.5	Active fluttering	Closed
8.5	Partly open with rapid closures	Slight fluttering (pulsation)
5.0	Wide open with occasional flutters	Active fluttering
4.5	Wide open with occasional flutters	Active fluttering
4.0	Wide open with occasional flutters	Wide open with occasional flutters
3.5	Wide open with occasional flutters	Wide open with occasional flutters
3.0	*Wide open with no closures*	Wide open with occasional flutters
2.5	*Wide open with no closures*	Wide open with occasional flutters
2.0	*Wide open with no closures*	*Wide open with no closures*
1.5	*Wide open with no closures*	*Wide open with no closures*

* Almost identical results were obtained when the gas mixtures were flushed through the chamber at random instead of in order, provided adequate recovery periods were allowed. Hence, under the conditions of our experiments, previous exposure to a test gas mixture had no effect on subsequent tests.

reason was that, in the normal intact insect, gas changes are ordinarily gradual and not cataclysmic, and hence it seemed a sensible procedure to allow time for adjustment to each change of gas.

Table V illustrates the data recorded to determine the effects of oxygen on two spiracles of a typical diapausing pupa. Progressive lowering of the ambient oxygen tension caused in sequence: pulsation of the valve (no visible opening), fluttering (small openings), partial openings and closings, full opening and occasional closure, and finally full opening with no movement, *i.e.*, complete relaxation of the spiracular closer muscle.

TABLE VI

"O_2-flutter" thresholds and "O_2-open" thresholds of abdominal spiracles of five intubated Cecropia pupae

Experiment No.	Spiracle observed	"O_2-flutter" threshold* (per cent)	"O_2-open" threshold** (per cent)
21 b	1 L	12	3
	3 L	8.5	3
22 b	1 L	12	1.5
	2 L	12	1.5
23 b	2 L	8.5	1.5
	3 L	5.5	1.5
24 b	1 L	12	1.5
	2 L	8.5	1.5
25 b	2 L	5.5	1.5
	3 L	5.5	1.5

* P_{O_2} which caused first pulsations.
** P_{O_2} which caused full spiracular opening for one minute.

Two oxygen tensions are of special interest: the tension that initiates spiracular movement, *i.e.*, pulsations or fluttering, and the tension that causes a spiracle to open fully, as in a burst. A typical set of values for these two oxygen tensions for a series of diapausing pupae is recorded in Table VI. From the table it can be seen that the five pupae studied varied considerably in their sensitivity to oxygen. Spiracular movements began at oxygen tensions ranging from 12 to 5.5%. The tensions required for full opening were more uniform. Unfortunately, the oxygen tension that induced pulsations proved to be exceedingly difficult to determine since it was not reproducible in successive experiments. The oxygen tension that produced full opening of the spiracle was far simpler to ascertain. Therefore, to facilitate quantitative measurements we concentrated on one spiracular response: full opening of the valve for one minute. This response occurs normally during a burst cycle, was easily judged, and was altered by changes in oxygen or carbon dioxide tensions of ± 0.5%. Moreover, it was easily reproducible.

The tension of oxygen necessary to open the spiracles widely was determined for 33 spiracles of more than a dozen Cynthia pupae that had several spiracles intubated. This tension of oxygen, hereafter termed the "O_2-open threshold," averaged 2.58 ± 1.12(s.d.)% with a range of 1% to 5.5%. Two-thirds of the spiracles responded to between 2% and 3.5% oxygen. Within any pupa the O_2-open threshold for

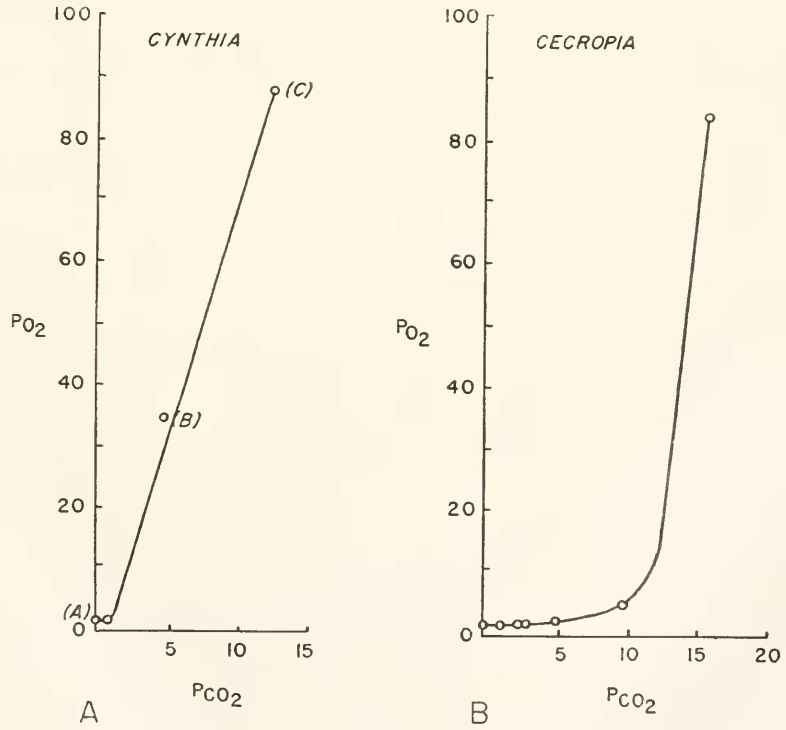

FIGURE 10. (A) The effect of P_{O_2} on the P_{CO_2} necessary to open a spiracle of an intubated Cynthia pupa widely for one minute. Partial pressures are expressed as % atm. For details see text. (B) Same curve as above for an intubated Cecropia pupa.

different spiracles did not vary by more than 1.5%. For a given spiracle, at all oxygen tensions below threshold the spiracle was wide open and the spiracular muscle remained relaxed; at tensions 0.5% or more above the O_2-open threshold, intermittent to prolonged contractions of the muscle occurred, which closed the valve (*cf.* Table V). The O_2-open threshold was also determined for a few developing adults of Polyphemus and found to average 3.1% oxygen with a range of 2.0–4.5%.

3. *The interaction between oxygen and carbon dioxide in the spiracles of intubated animals*

The carbon dioxide tension necessary to open the spiracle widely for one minute varied with the oxygen tension. This is seen in Figure 10A which records the behavior of a Cynthia pupa. The first point (A) on the curve gives the highest partial pressure of oxygen in a nitrogen-oxygen mixture that kept the spiracle open, *i.e.,* the O_2-open threshold which we considered in the previous section. The third point (B) on the curve was determined by adding a mixture of 4% carbon dioxide to air, a mixture which kept the spiracle wide open, and progressively increasing the oxygen tension until the spiracle began to close. In this experiment, in the presence of 4% carbon dioxide, oxygen tensions above 35% were necessary to close the spiracle. In other words at any point along the curve for a given partial pressure of carbon dioxide there was a critical tension of oxygen, below which the valve would remain fully open and motionless; oxygen tensions above this critical tension caused closure of the valve. The end-point of this curve is (C). At carbon dioxide tensions above this value the valve remains open and motionless and no partial pressure of oxygen at atmospheric pressure will cause fluttering. This point, which is designated the "CO_2-open threshold," was determined by starting in pure oxygen where the valve was closed and increasing the carbon dioxide tension until a tension was reached at which the spiracle remained open for one minute. This CO_2-open threshold was simple to determine and reproducible within about 1%. Several curves of this sort were recorded for a number of pupae and all looked essentially the same. One typical of a Cecropia pupa is presented in Figure 10B.

Although such curves are difficult to obtain, by measuring only the O_2-open threshold and the CO_2-open threshold of a spiracle it was possible to assess in a general way the sensitivity of the spiracle and to compare spiracles of pupae under various conditions. This was done for a series of Cynthia spiracles and the O_2-open thresholds have already been discussed. The CO_2-open thresholds of this same series of spiracles averaged 17.63 ± 5.42(s.d.)% and covered a far greater range (13.5% to 31%) than the O_2-open thresholds. Notwithstanding, two-thirds of the spiracles had a CO_2-open threshold between 14% and 16%. The CO_2-open threshold appeared quite uniform for different spiracles of any individual. A few determinations were also made on the spiracles of developing adults of Polyphemus. Whereas the O_2-open thresholds for these insects with high metabolic rates did not differ significantly from the thresholds for diapausing pupae, the CO_2-open thresholds of the developing adults were lower than for the diapausing pupae and averaged only 10.5% with a range of 9.5–12.0%. Furthermore, the CO_2-open thresholds decreased on successive days of adult development as the metabolic rate increased.

These results demonstrate the influence of oxygen on the response of the spiracle to carbon dioxide, and indicate that the sensitivity to carbon dioxide decreases as oxygen tension increases. Similar results were obtained when the carbon dioxide tension that caused fluttering was determined: the sensitivity to carbon dioxide decreased as the oxygen tension increased.

Discussion

1. *Scope of the analysis*

The spiracular behavior of giant silkworms just described represents one of the most extreme kinds of spiracular behavior recorded for any insect, with a complex cycle of activity which commonly lasts for hours and occasionally for days. The present experiments confirm and amplify earlier opinions that the cyclical behavior of the spiracles is somehow responsible for discontinuous respiration. They may have, in addition, some intrinsic interest for insect respiration as a whole. For, in a sense, these giant pupae breathe in "slow motion," and events which may occupy only a second or two in the respiratory cycles of other insects may take many minutes or even hours. This enables the experimenter to analyze complex events in gas exchange and spiracular behavior which are not readily separable in insects with brief cycles. The present experiments have exploited this property in an effort to define the kinds of behavior that spiracles can show, and to clarify the manner in which oxygen and carbon dioxide interact to provoke various modes of spiracular activity.

2. *The behavior of the spiracular muscle*

Interpretation of the present experiments is simplified by the fact that the movements of each pupal spiracle are controlled by a single muscle. Thus, the records of spiracular activity are records of the behavior of this muscle. As we have seen, the muscle displays three sorts of behavior: sustained contraction which causes valve closure; brief partial relaxation which causes valve fluttering; and almost complete relaxation which causes the valve to gape open.

In the normal pupa these different kinds of behavior occur in two characteristic patterns: (1) the first is the "flutter," in which the muscle relaxes slightly and then quickly re-contracts fully. This occurs many times each minute in irregular volleys and may continue for hours. It is important to note that the relaxations are very brief and that during most of the flutter period the valves are constricted. Indeed, as far as one can tell, they are as tightly constricted between flutters as they are during the constriction period after a burst. (2) The second pattern, the "burst" cycle, is more dramatic and far more regular. It is compounded of numerous flutters and periods of prolonged valve opening and prolonged constriction.

The initiation of each of these patterns of spiracular behavior does not appear to depend upon an endogenous nervous rhythm as is true, for example, in the respiratory movements of *Schistocerca* and *Dixippus* (*cf.* review by Wigglesworth, 1953a, and also Hoyle, 1959) or of vertebrates, but is the result of cyclical changes in the composition of the tracheal gases. The mechanism of this extrinsic control is described below.

3. *An explanation for the burst cycle*

All events in the normal burst cycle, as well as the experimental observations, can be explained in terms of the following three properties of the spiracular muscle and its associated nerves: (a) the muscle is ordinarily continuously stimulated to contract; (b) when tracheal P_{O_2} falls below a critical value, the flutter threshold, the muscle repeatedly relaxes slightly and re-contracts; (c) when tracheal P_{CO_2} rises above a critical value, the burst threshold, the muscle relaxes fully. These assertions permit us to frame the following hypothesis which appears to be consistent with all of our observations and accounts for the burst cycle.

Immediately after a burst the valves are constricted, tracheal P_{O_2} is far above the flutter threshold, and tracheal P_{CO_2} is far below the burst threshold. This is the constriction period. As the insect respires, oxygen inflow fails to match oxygen utilization and the P_{O_2} within the tracheal system falls. When the tracheal P_{O_2} falls to about 3%, the flutter threshold, spiracular fluttering begins, permitting oxygen to enter. This increases the tracheal P_{O_2} and the valve promptly closes. This pattern of rapid fluttering, punctuated by short periods of constriction, represents the flutter period. In high oxygen tensions tracheal P_{O_2} never reaches triggering concentration and hence no fluttering occurs.

What about the spiracular burst itself? This involves carbon dioxide. During the interburst period some carbon dioxide escapes, but at a rate considerably less than that of its production. As a consequence, internal P_{CO_2} ultimately reaches the spiracular burst threshold, and the spiracular muscle relaxes. The valve opens widely in a burst; carbon dioxide and nitrogen flow out and oxygen flows in, as the composition of the tracheal gas approaches that of the atmosphere. When the internal P_{CO_2} decreases below a certain value, the valve begins to open and close periodically, with diminishing width and duration of opening, until it finally remains constricted and the cyle is completed.

Detailed support for this hypothesis and for the assertions regarding the effect of P_{O_2} and P_{CO_2} on the spiracular muscle are examined below.

4. *Oxygen effects: the constriction and flutter periods*

A number of experiments prove that fluttering is caused by low tracheal P_{O_2} and is little affected by tracheal P_{CO_2}. Firstly, in a normal pupa, lowering ambient P_{O_2} to about 10% evokes continuous valve fluttering (presumably because tracheal P_{O_2} is lowered). Secondly, raising ambient P_{O_2} suppresses entirely the valve fluttering of a normal pupa: apparently under this condition tracheal P_{O_2} never falls to the flutter threshold. Thirdly, when several spiracles are intubated, thereby raising the internal P_{O_2}, fluttering ceases. Finally, in such an intubated pupa, when ambient P_{O_2} is lowered to about 2% fluttering continues indefinitely.

Under this analysis, in a normal pupa the *duration of the constriction period* after a burst is determined primarily by the rate at which P_{O_2} falls within the tracheal system. This in turn depends upon three factors: (1) the tracheal volume of the pupa, (2) the rate of oxygen utilization by the pupa, and (3) the rate at which oxygen leaks in through the constricted spiracles. In general, large pupae with low metabolic rates have long constriction periods. Also, as one would predict, the constriction period is prolonged by increasing ambient P_{O_2} (*cf.* Table III),

for at the end of a burst, not only is there a higher O_2 content in the tracheal system, but also more O_2 leaks inward.

In occasional experiments, pure oxygen failed to suppress valve fluttering but instead abolished the burst cycle. Similar results were found by Buck *et al.* (1955) in manometric experiments with *Agapema* pupae. Neuromuscular mechanisms of insects are particularly susceptible to oxygen poisoning (Goldsmith and Schneiderman, 1960) and this may account for these results.

What determines the frequency of fluttering and the duration of the closed period between each volley of flutters? Both of these appear to be governed, as is the duration of the constriction period, by the rate at which P_{O_2} falls within the tracheal system, and therefore depend in part upon metabolic rate and upon ambient P_{O_2}. As one would expect, the higher the metabolic rate, the more frequent the flutters and the more frequent the volleys of flutters (*cf.* Figures 4 and 6). Moreover, since the frequency of the flutters and the frequency of volleys depend primarily upon tracheal P_{O_2}, it is not surprising that they do not change systematically during the course of the flutter period, for *once fluttering begins, tracheal P_{O_2} presumably varies very little since oxygen continuously enters.* This prediction has been confirmed by analyses of tracheal gas composition (Levy and Schneiderman, 1957, 1958), which will be discussed in detail in a subsequent report. For our present purposes this constant P_{O_2} is of singular importance, for it tells us that the termination of the flutter period by a spiracular burst depends not upon a change in tracheal P_{O_2}, but upon the accumulation of carbon dioxide. This is considered below.

5. *Carbon dioxide effects: "triggering" and termination of the spiracular burst*

The most conspicuous effect of an increase in ambient P_{CO_2} was the shortening of the burst cycle, which confirms previous manometric findings (Schneiderman and Williams, 1955). This reduction in cycle length was largely at the expense of the flutter period, which was greatly shortened, whereas the duration of the constriction period was unchanged. In short, increasing ambient P_{CO_2} led to the premature triggering of the burst early in the flutter period. Moreover, once triggered, the burst was prolonged. These observations can be understood in the following terms:

(1) *The absence of an effect of carbon dioxide on the duration of the constriction period* supports the opinion offered above, that in the intact pupa the onset of fluttering is triggered primarily by low tracheal P_{O_2}. Further support comes from the fact that the P_{O_2} which triggered off the fluttering was not significantly affected by modest increases in tracheal P_{CO_2}. Indeed, in pupae with long cycles, until ambient P_{CO_2} rose above 10%, it did not appear to affect the amplitude or frequency of flutters.

(2) *The premature triggering of spiracular bursts by ambient P_{CO_2}.* A variety of experiments revealed that in a normal burst cycle, the spiracular bursts themselves are triggered by tracheal P_{CO_2}, and not by tracheal P_{O_2}. Thus exposing both normal and intubated pupae to physiological and non-anaesthetic concentrations of carbon dioxide causes the spiracular valves to open widely. In a normal cycle, tracheal P_{CO_2} gradually increases after a burst as a result of metabolism and, when it reaches a certain level, the spiracular muscle fully relaxes and a burst occurs. At *all* concentrations of carbon dioxide, tracheal P_{CO_2} more quickly reaches the

triggering concentration, because the partial pressure gradient driving carbon dioxide out of the insect is decreased, with a consequent decrease in the rate of carbon dioxide leakage. In addition, *high* ambient concentrations of carbon dioxide (*i.e.*, above 5%) prevent tracheal carbon dioxide from falling to its normal low level after a burst. This constant high level of tracheal carbon dioxide also shortens the time required to reach a trigger value. Thus, for these two reasons, the higher the ambient carbon dioxide concentration, the shorter the time between bursts. The second reason is by far the more important in pupae with low metabolic rates. In such insects ambient carbon dioxide tensions below 5% had no detectable effect on burst frequency, whereas increasing carbon dioxide from 5% to 10% often increased the burst frequency 10 to 20 times (Schneiderman and Williams, 1955). However, in pupae with high metabolic rates and high burst frequencies, the reduction in carbon dioxide escape caused by the presence of even 1% ambient carbon dioxide was often sufficient to abolish the bursts entirely.

A different kind of evidence for carbon dioxide triggering spiracular bursts comes from experiments on *Agapema* pupae, in which there was demonstrated an inverse relation between ambient P_{CO_2} and the *delay* in the first subsequent spiracular opening (Buck and Keister, 1958). Pupae in 9% carbon dioxide opened their spiracles $2\frac{3}{4}$ hours after exposure, whereas in 24% carbon dioxide they took only one hour. As the authors point out, this is what would be expected "if the carbon dioxide were leaking into the tracheal system and accelerating the rise to triggering concentration" (p. 335).

(3) *Further evidence for the triggering of spiracular bursts by tracheal* P_{CO_2}. The triggering of bursts by tracheal P_{CO_2} is well seen in 100% oxygen, where internal P_{O_2} might be expected to remain relatively high throughout the cycle. Here it seems clear that increasing tracheal P_{CO_2} was the trigger that caused the spiracular muscle to relax. The converse result is seen in intubated pupae, where one prevents carbon dioxide from reaching a concentration high enough to trigger a spiracular burst and the valves remain permanently closed.

(4) *Termination of the spiracular burst.* Once triggered to open in a burst, the spiracle remains open and carbon dioxide diffuses out until tracheal P_{CO_2} falls to some critical level, whereupon the spiracle closes again. Increased ambient P_{CO_2} prolonged bursts, presumably because it delayed the diffusive outflow of carbon dioxide. It is worth recalling that it takes the blood and tissues some time to unload carbon dioxide. In most cases, long before this is done the tracheal P_{O_2} has risen to its maximum value as oxygen diffuses in at the burst. Hence, the closing of the spiracle at the end of a burst has much less to do with tracheal P_{O_2} than with tracheal P_{CO_2}.

It is puzzling that immediately the carbon dioxide tension falls somewhat, the muscle does not contract again, but instead remains relaxed for many minutes, even when carbon dioxide tension has fallen well below the triggering threshold. In some way, high P_{CO_2} provokes a response in the muscle which is sustained for a considerable period (*cf.* discussion of this point by Buck, 1957). This sharply contrasts with the partial relaxations induced by low P_{O_2}, which are very transitory, and suggests that flutters and spiracular bursts are fundamentally different responses.

6. *Effects of metabolic rate*

The effects of metabolic rate on spiracular activity appear to be similar in most insects: high metabolism leads to increased spiracular movement (*cf.* review by Wigglesworth, 1953a). For example, Nunome (1944) has reported that the spiracles of *Bombyx mori* open more frequently and more widely when larvae were active than when they were inactive. In the present experiments, spiracular burst frequency increased with metabolic rate, confirming manometric studies. This increase in frequency is largely the result of more rapid production of carbon dioxide which shortens the time required for tracheal P_{CO_2} to reach triggering concentration. The more rapid decrease in tracheal P_{O_2} which attends high metabolism probably has only a limited impact, inasmuch as once fluttering begins, tracheal P_{O_2} remains constant.

When metabolic rate gets very high and bursts occur every 5 or 6 minutes (see for example Figure 6), the constriction period is obliterated, and between bursts the valves are in continuous motion, *i.e.*, fluttering. In effect, the decline period after the burst fuses with the flutter period before the next burst. This suggests that oxygen utilization is so rapid that even during the decline period tracheal P_{O_2} falls rapidly to the flutter threshold. When metabolic rate gets very high and the cycles last for less than four minutes, as is the case with late developing adults, the interburst flutterings increase markedly in amplitude until the burst cycles disappear entirely and are replaced by continuous wide flutters. As mentioned previously, these flutters are far larger than oxygen-induced flutters. It appears likely that when metabolism gets high enough to cause continuous fluttering, carbon dioxide may be viewed as the principal stimulus. Further support for this opinion comes from the observation that in intubated pupae the O_2-open threshold did not vary with metabolic rate, whereas the CO_2-open threshold decreased as metabolic rate increased. This suggests that in pupae with high metabolic rates, there is a considerable accumulation of carbon dioxide even when several spiracles are open. This high tracheal P_{CO_2} probably accounts for the fact that intubated pupae with very high metabolic rates showed continuous valve fluttering.

7. *Interaction between carbon dioxide and oxygen*

In the above analysis we have considered oxygen and carbon dioxide as acting essentially independently. This is not the case, but, as we shall see, this fact does not impair the analysis. Evidence that oxygen tension affects the carbon dioxide threshold was provided by the observation that high oxygen tension markedly reduced the burst frequency from 5 to 10 bursts per hour to one to two per hour in a pupa in which 5 pairs of spiracles were sealed. This finding suggests that the high oxygen tension raised the carbon dioxide threshold so that more carbon dioxide had to accumulate to cause a spiracular burst. A further piece of circumstantial evidence was the discovery that very high oxygen tensions often restored bursts in pupae in which carbon dioxide had eliminated the bursts. However, interpretation of these experiments was hampered by the fact that it was difficult to be certain of the tracheal gas composition, which varied continuously. Part of this difficulty was by-passed in experiments with intubated pupae. To be

sure, even in intubated pupae the tracheal gas composition is not known with certainty, but at least it can be kept constant and the oxygen and carbon dioxide tensions can be varied independently.

The intubation experiments reveal that the concentration of carbon dioxide necessary to open the spiracles wide varies with the oxygen tension. In the presence of 2.3% oxygen, 5% carbon dioxide opened the spiracles of a typical Cecropia pupa, whereas in 4.7% oxygen, 10% carbon dioxide was required. This result demonstrates clearly the interaction between P_{O_2} and P_{CO_2} in controlling the wide opening of spiracles, and it finds several parallels in the literature. Thus, in the cockroach (Hazelhoff, 1926a, 1926b), the flea (Wigglesworth, 1935), and in various muscid flies (Case, 1956a), the opening of spiracles in response to carbon dioxide is favored by low oxygen and depressed by high oxygen, just as in Cynthia and Cecropia pupae.

In the present experiments it is of some interest that, although P_{O_2} influenced the P_{CO_2} necessary to cause a spiracular burst, there did not seem to be a similar interaction in the triggering of flutters. Thus, as discussed in Section 5 above, increasing ambient P_{CO_2} up to 10% had no significant effects on the oxygen flutter threshold. Hence, it seems unlikely that during a normal burst cycle internal P_{CO_2} affects the oxygen flutter threshold, inasmuch as tracheal P_{CO_2} in these pupae rarely rises above 6.5% (Buck and Keister, 1958; Levy and Schneiderman, 1958).

It is not our present purpose to explore the mechanism of the interaction between oxygen and carbon dioxide in triggering bursts. However, recognition of this phenomenon enables us to interpret several events in the burst cycle to which we have not yet attended, notably the onset of the decline period. Why does the relaxed spiracular muscle start contracting again about one-third of the way through a burst? This might be the result of falling tracheal P_{CO_2}, rising tracheal P_{O_2}, or both. Evidence that it is largely rising P_{O_2} comes from records of pupae in pure oxygen. Here there is no decline period and the spiracle simply shuts. This suggests that under normal conditions the decline period is related to tracheal P_{O_2}. A possible mechanism might be the following.

During a spiracular burst, tracheal P_{O_2} equilibrates with the atmosphere far more rapidly than does tracheal P_{CO_2} because of the long time required to unload dissolved carbon dioxide. In a relatively short time, tracheal P_{O_2} rises to a concentration that increases the carbon dioxide burst threshold. The valve shuts briefly, to reopen promptly as carbon dioxide continues to accumulate in the tracheal system. Under this analysis, the onset of the decline period marks the point in a spiracular burst where tracheal P_{O_2} has risen high enough to depress appreciably the sensitivity of the spiracle to carbon dioxide. As mentioned in Section 5 above, the duration of the decline period is determined by the time necessary to unload carbon dioxide.

A second instance of O_2-CO_2 interaction appears to occur in the period just before a spiracular burst. Ordinarily, 20 to 120 seconds before a burst, flutters are larger than normal and gradually build up to the burst. This brief build-up period resembles the decline period and is also absent in pure oxygen, suggesting that, like the decline period, it reflects O_2-CO_2 interaction. The mechanism of this interaction will be considered subsequently, but for the present we can conclude that at the low P_{O_2} which obtains in a normal pupa just prior to a burst, the

carbon dioxide threshold of different excitatory elements is not as uniform as in high P_{O_2}.

Interaction between oxygen and carbon dioxide also explains why variations in ambient P_{O_2} have no predictable effect on burst frequency. High ambient P_{O_2} raises the carbon dioxide threshold of the spiracles, and this tends to decrease the burst frequency. But, simultaneously, high oxygen decreases interburst fluttering and this, in turn, decreases the rate of interburst carbon dioxide release, and increases the rate of carbon dioxide accumulation. This tends to increase the burst frequency. The ultimate outcome, as we know from manometric experiments (Schneiderman and Williams, 1955) and from the present study, is sometimes increased frequency, sometimes decreased frequency, and in other cases no change.

Table VII summarizes the conclusions reached in this discussion relating to the effects of tracheal P_{O_2} and P_{CO_2} on the behavior of the spiracular valves during the burst cycle.

TABLE VII

Summary of the effects of tracheal P_{O_2} and P_{CO_2} on the behavior of the spiracular valves during a burst cycle

Tracheal composition	Constriction period		Flutter period		Burst	
	Initiate	Terminate	Initiate	Terminate	Initiate	Terminate
Low P_{CO_2}	+	0	0	0	0	+
High P_{CO_2}	0	(+)	(+)	+	+	0
Low P_{C_2}	0	+	+	(+)	(+)	0
High P_{O_2}	±	0	0	0	0	±

\+ Principal agency responsible.
± Secondary factor.
(+) These conditions do not normally prevail in the phase of the burst cycle indicated.

8. *The existence of an independent oxygen-sensitive mechanism*

It is not a principal purpose of the present report to discuss the mechanisms whereby oxygen and carbon dioxide exert their effects, nor to consider the sites of action of these gases. Earlier experiments on these pupae ruled out the existence of any indispensable respiratory center (Schneiderman and Williams, 1953b; Schneiderman and Beckel, 1954; Schneiderman, 1956). They also showed that although nervous control was involved in the normal stimulation and coordination of the spiracular muscles (Schneiderman, 1956), the immediate response to carbon dioxide resided in the spiracular muscle itself (Beckel and Schneiderman, 1956, 1957). However, the present studies appear to clarify one point which has hitherto not been resolved for any insect, namely that, at least in silkworm pupae, *oxygen and carbon dioxide affect the spiracle via very different mechanisms.* It has been suggested by various workers that oxygen-lack produces acidity, and this might be its mode of action in stimulating spiracles. Wigglesworth (1935, 1953b) argues that in the flea both O_2-lack and CO_2-excess act in virtue of the

acidity they produce. Case (1957a) and Punt *et al.* (1957), on the other hand, raise the possibility that the acidity produced by oxygen-lack might release bound carbon dioxide from the blood and that CO_2 is the effective agent to which spiracles respond in both O_2-lack and CO_2-excess. In the case of the silkworm, the experimental results forbid either interpretation, as the following considerations show.

During the course of a burst cycle, when P_{O_2} falls to a critical threshold, fluttering begins and may continue uniformly for many hours. This sustained pattern of behavior in response to low P_{O_2} indicates that the primary response to oxygen lack is not acidity itself, nor acidity releasing bound carbon dioxide. For, during the entire course of the flutter period, acidity increases steadily as carbon dioxide accumulates. Notwithstanding, the flutter response did not change appreciably, nor did it change after ambient P_{CO_2} was raised to 10% (*cf.* Figure 8 and Section 5 above), a maneuver which certainly increased acidity. These observations suggest that, in the silkworm, increasing acidity is probably not the means whereby oxygen lack makes itself felt. They also seem to prove that in the silkworm pupa there is a specific mechanism sensitive to oxygen lack that may act independently of any carbon dioxide-sensitive mechanism.[4] Evidence will be presented in a subsequent paper that P_{O_2} may act on the central nervous system in contrast to P_{CO_2} which acts peripherally.

9. *Comparison with the flea*

In concluding, it is fruitful to compare the spiracular behavior of these silkworm pupae with the behavior of the spiracles of the flea described by Wigglesworth (1935) in his fundamental study. The flea spiracle is operated by a single closer muscle and shows a simple pattern of opening and closing. In air at 20 to 22° C. cycles are 12 to 16 seconds long, 6 to 8 seconds during which the spiracle stays closed followed by about an equal time during which the spiracle is open. P_{O_2} and P_{CO_2} affected this cycle in a predictable way. As P_{O_2} diminished from 100% there was an increase in cycle frequency; the closed period was considerably shortened, whereas the open period was less affected. As P_{CO_2} increased, the open period was lengthened considerably, whereas the closed period was slightly shortened. It was concluded that the spiracles were caused to open chiefly by falling P_{O_2}, rising P_{CO_2} being less important.

In the pupal burst cycle, the constriction period corresponds to the closed period in the flea, and the spiracular burst corresponds to the open period in the flea. Because the flea has no flutter period we cannot compare the effects of various gas mixtures on the length of an entire cycle (*i.e.*, on the frequency of the cycles), but we can compare the periods of valve closure and of valve opening in both insects.

(a) *Oxygen effects*

In both the flea and the pupa the duration of the constricted period is proportional to P_{O_2}, and in both, the spiracle is caused to open by low P_{O_2}. However,

[4] Very recently Hoyle (1960) has provided convincing evidence that in the spiracular muscle of the locust, CO_2 acts directly on the neuromuscular junction and blocks transmission of nervous excitation. This important discovery supports the view outlined above that CO_2 acts peripherally.

unlike the flea spiracle, which opens widely and stays open for a number of seconds after a constriction period, the pupal spiracular muscle relaxes only slightly when a critical P_{O_2} is reached and then re-contracts immediately. This behavior is repeated over and over during the flutter period, for which there is no counterpart in the flea. In the pupa, for reasons already discussed in Section 7, P_{O_2} has no predictable effect on cycle frequency.

(b) *Carbon dioxide effects*

In both the flea and the pupa the duration of the spiracular burst or open period is proportional to P_{CO_2}. And in both, P_{CO_2} has only small effects on the duration of the constriction period. It is noteworthy that in the flea, carbon dioxide fails to affect the frequency of openings and in the pupa it fails to affect the frequency of flutters or volleys of flutters in the flutter period. These observations support the view that the open period of the flea and the flutter period of the pupa are largely triggered by low tracheal P_{O_2}, and that tracheal P_{CO_2} is ordinarily of much less importance in this connection. It is nonetheless of some significance that, in the flea, raising the P_{CO_2} decreased the closed period, albeit only slightly. In commenting upon this, Wigglesworth (1935) remarks (p. 405) that the closed period of the flea is terminated "chiefly by oxygen want (carbon dioxide contributing to a small extent)." The pupal spiracular burst cycle enables us to separate clearly the oxygen-want effect from the carbon dioxide effect, for in the pupa the oxygen-want effect—fluttering—not only appears first, but is qualitatively different from the carbon dioxide effect—the initiation of a spiracular burst. In only one place in the flea are these effects separable, namely, in pure oxygen. Here, in the flea as in the pupa, the spiracular bursts seem to have been triggered mainly by P_{CO_2} and not by oxygen lack. It is also noteworthy that in both the flea and the pupa, the effects of carbon dioxide varied directly with the intensity of metabolism.

A further point of similarity between the flea and the pupa is that in both, the denervated spiracular muscle remains contracted (Wigglesworth, 1935; Schneiderman, 1956). In the case of the pupal silkworm the muscle remains contracted for many weeks, and this appears to be true also of the bed-bug (Wigglesworth, 1941) and the roach (Case, 1956b, 1957b). These are all insects without nervous-controlled ventilation movements under ordinary conditions. By contrast, the spiracular muscles of insects like *Schistocerca*, which have ventilation movements, relax when denervated (Fraenkel, 1932; Hoyle, 1959). Indeed, it may be that in many insects where the rhythmical activity of spiracles is under nervous control, denervation leads to relaxation, whereas in others, like the flea and silkworm pupae, where the spiracles are under the extrinsic control of respiratory gases, the denervated spiracular muscle continues to contract.

In sum, it appears that the behavior of the spiracles of both the flea and the pupa is remarkably similar. A crucial difference between the two respiratory cycles is the flutter period of the pupa. This cannot stem simply from differences in the intensity of metabolism, for when fleas are placed at temperatures as low as 4° C., which reduces their metabolism to levels characteristic of diapausing pupae, they do not show flutters. Similarly, raising the metabolism of pupae to that of fleas by injury or by other means fails to produce the flea pattern of pro-

longed openings. The reason for the different modes of spiracular behavior is probably to be found, at least in part, in the size of the insects and the attendant differences in the lengths of both fluid and gaseous diffusion paths. Contributing also to the differences in spiracular behavior is the relative insensitivity of the O_2- and CO_2-sensitive mechanisms. This was recognized at the outset by Wigglesworth (personal communication), who pointed out that among the preconditions for discontinuous respiration was that the responding system "must have an extremely high threshold of stimulation by carbon dioxide . . . and this means that there must be a very large accumulation of CO_2 before it will cause the spiracles to open." This is surely so, and the CO_2 threshold of the flea is likely much lower than that of the pupa.

However, the differences between the spiracular behavior of the pupa and the flea are modest when weighed against the similarities. In summarizing his findings on the flea, Wigglesworth (1935) concluded (p. 402) that each respiratory act was "determined by an immediate stimulus of a chemical nature." The present experiments extend these conclusions to the silkworm and indicate that carbon dioxide bursts result in large measure from an exaggeration of a basically simple sort of spiracular behavior.

10. *Comparison with other insects*

When the spiracles of other insect groups are examined, it becomes immediately clear that fluttering, although not unique to silkworms, may be less common than the simple closing and opening observed in the flea. For example, Hazelhoff (1926a) reported no fluttering in any of the Diptera, Trichoptera, Neuroptera and Odonata that he examined. However, he says that in *Periplaneta americana* ". . . All the stigmata are almost closed. . . . The movable stigmal valve performs *usually quick vibrating movements*" (flutters) "whereby nevertheless the opening width of the stigmata remains generally quite small" (p. 70). He adds that ". . . the stigmata in pure O_2 are more closed than in ordinary air," which suggests that here, as in the silkworm, fluttering is controlled by P_{O_2}. Fluttering also occurs in locusts (Hoyle, 1959). How widespread fluttering is in other orders and whether it is usually controlled by P_{O_2} remains to be seen.

11. *Prospects*

The main function of spiracular closing mechanisms is water conservation. From an evolutionary viewpoint, we may imagine that natural selection has favored the development of mechanisms that keep spiracles open just enough to permit the exchange of respiratory gases but otherwise restrict their aperture. Such a water-conserving mechanism might be expected to be best developed in insects with severe water problems, like diapausing pupae which live for long periods without imbibing (*cf.* Imms, 1957, p. 145). As Buck points out (1957, p. 77) discontinuous respiration is an example of such a well-developed spiracular mechanism. And, bursts of carbon dioxide are easily understandable in terms of the spiracular movements, which have been analyzed in earlier sections of this discussion. But what of the disparate rates of gas exchange between bursts, which are the real enigma of discontinuous respiration? Somehow during the interburst the spiracles manage to let oxygen enter, yet simultaneously prevent carbon dioxide

and water vapor from leaving. Whilst the present experiments have not penetrated this secret, they nonetheless provide an experimental framework upon which a theory of mechanism must be based. For the heart of the problem surely lies in the flutter period, where hour after hour the insect practices the "trick" of filtering in oxygen, while retaining carbon dioxide and water. It is our belief that the real significance of the flutter period may have been overlooked in previous explanations of this peculiar one-way transfer of gases. The importance of the flutters in the kinetics of gas exchange will be discussed in a succeeding communication.

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Summary

1. Experiments were conducted to examine the role of the spiracles in discontinuous respiration, to define the kinds of behavior that spiracles can show, and to clarify the manner in which tracheal P_{O_2} and P_{CO_2} interact to provoke various modes of spiracular activity. To accomplish this, records were made of the movements of the spiracular valves of diapausing pupae and developing adults of the Cecropia, Polyphemus and Cynthia silkworms, in air and in gas mixtures.

2. Spiracular valve movements in these silkworms occur in repeated cycles, with periods of from a few minutes to many hours. Each cycle consists of an open period or spiracular burst (which corresponds to the CO_2 burst), a closed or constriction period, and a flutter period which ordinarily occupies most of the cycle. In pupae with long cycles, the respiratory events occur virtually in slow motion when compared with other insects, and this permits careful analysis of complex events in gas exchange and spiracular behavior which are not readily separable in other insects. Evidence is presented that each spiracular act (fluttering, burst, valve closure) is a response to a specific chemical stimulus: the gaseous composition of the tracheal system.

3. In pure O_2, the flutter period is ordinarily eliminated. As ambient P_{O_2} decreases, fluttering reappears and the flutter period progressively lengthens, until, in P_{O_2}'s below 15%, the spiracular bursts disappear and fluttering is continuous.

4. Ambient P_{CO_2} ordinarily has no effect until it increases above 5%, whereupon the cycles shorten. This shortening occurs at the expense of the flutter period, which progressively diminishes as P_{CO_2} increases. When P_{CO_2} rises above about 15%, the cycles break down completely and the valves flutter continuously.

5. Intubating even one pupal spiracle eliminates the cycles in the remaining 13, and the valves stay permanently constricted, presumably because normal triggering stimuli for spiracular activity are absent.

6. The spiracles of intubated pupae can be caused to open by lowering the P_{O_2} or raising the P_{CO_2}. The P_{CO_2} which opens the spiracles varies with the ambient P_{O_2}: in a typical pupa, in 2.3% O_2, 5% CO_2 opened the spiracles, whereas in 4.7% O_2, 10% CO_2 was required.

7. From these and other data, it is concluded that the cyclical movements of the spiracles result from cyclical changes in tracheal composition. In particular, fluttering is initiated by low P_{O_2}, while spiracular bursts are caused by high P_{CO_2}.

8. Evidence is presented to prove that the pupa possesses an independent O_2-sensitive mechanism, which is quite separate from any CO_2-sensitive mechanism. It is also argued that low P_{O_2} does not affect spiracular behavior by virtue of anoxia-produced acidity, nor by virtue of acidity releasing bound carbon dioxide.

9. The spiracular behavior of these silkworms is compared in detail with the picture of spiracular behavior in the flea provided by Wigglesworth, and it is concluded that there is no fundamental difference between the two, except for the flutter period which seems peculiar to the pupa. Evidence is presented that the flutter period holds the key to the disparate rates of gas exchange between bursts, which remains the central problem of discontinuous respiration.

LITERATURE CITED

Beckel, W. E., 1955. The morphology, histology, and physiology of the spiracular regulatory apparatus of *Hyalophora cecropia* (L.) (Lepidoptera). Doctoral thesis, Cornell University.

Beckel, W. E., 1958. The morphology, histology and physiology of the spiracular regulatory apparatus of *Hyalophora cecropia* (L.) (Lepidoptera). *Proc. 10th Int. Congress of Entomol.*, **2**: 87–115.

Beckel, W. E., and H. A. Schneiderman, 1956. The spiracle of the Cecropia moth as an independent effector. *Anat. Rec.*, **125**: 559–560.

Beckel, W. E., and H. A. Schneiderman, 1957. The insect spiracle as an independent effector. *Science*, **126**: 352–353.

Buck, J. B., 1957. Triggering of insect spiracular valves. Pp. 72-79 in "Physiological Triggers," ed. by T. H. Bullock, Amer. Physiol. Soc., Washington, D. C.

Buck, J. B., 1958a. Possible mechanism and rationale of cyclic CO_2 retention by insects. *Proc. 10th Int. Congress of Entomol.*, **2**: 339–342.

Buck, J. B., 1958b. Cyclic CO_2 release in insects. IV. A theory of mechanism. *Biol. Bull.*, **114**: 118–140.

Buck, J. B., M. Keister and H. Specht, 1953. Discontinuous respiration in diapausing *Agapema* pupae. *Anat. Rec.*, **117**: 541.

Buck, J. B., and M. Keister, 1954. Spiracular retention of CO_2 without O_2 limitation. *Anat. Rec.*, **120**: 731.

Buck, J. B., and M. Keister, 1955. Cyclic CO_2 release in diapausing *Agapema* pupa. *Biol. Bull.*, **109**: 144–163.

Buck, J. B., and M. Keister, 1958. Cyclic CO_2 release in diapausing pupae—II. Tracheal anatomy, volume and P_{CO_2}; blood volume; interburst CO_2 release rate. *J. Ins. Physiol.*, **1**: 327–340.

Case, J. F., 1956a. Carbon dioxide and oxygen effects on the spiracles of flies. *Physiol. Zoöl.*, **29**: 163–171.

Case, J. F., 1956b. Spontaneous activity in denervated insect muscle. *Science*, **124**: 1079–1080.

Case, J. F., 1957a. Differentiation of the effects of pH and CO_2 on spiracular function of insects. *J. Cell. Comp. Physiol.*, **49**: 103–114.

Case, J. F., 1957b. The median nerves and cockroach spiracular function. *J. Ins. Physiol.*, **1**: 85–94.

Fraenkel, G., 1932. Untersuchungen über die Koordination von Reflexen und automatisch-nervösen Rhythmen bei Insekten III. Das Problem des gerichteten Atemstromes in den Tracheen der Insekten. *Zeitschr. vergl. Physiol.*, **16**: 418–443.

Goldsmith, M. H. M., and H. A. Schneiderman, 1960. The effects of oxygen poisoning on the post-embryonic development and behavior of a chalcid wasp. *Biol. Bull.*, **118**: 269–288.

HAZELHOFF, E. H., 1926a. Over een nieuwen vorm van ademhalingsregeling (diffusie regeling) bij insecten en spinnen. Thesis, Utrecht. (Complete English translation from National Institutes of Health, courtesy Dr. John B. Buck). *Cf.* also Jordan, 1927, *Zeitschr. vergl. Physiol.*, **5**: 179–190.

HAZELHOFF, E. H., 1926b. On a new form of breathing regulation (regulation of diffusion) in Insects and Arachnida. *Proc. Sect. Sci. Koninkl. Acad. Wetensch. Amsterdam*, **29**: 492–496.

HERFORD, G. M., 1938. Tracheal pulsation in the flea. *J. Exp. Biol.*, **15**: 327–338.

HOYLE, G., 1959. The neuromuscular mechanism of an insect spiracular muscle. *J. Ins. Physiol.*, **3**: 378–394.

HOYLE, G., 1960. The action of carbon dioxide gas on an insect spiracular muscle. *J. Ins. Physiol.*, **4**: 63–79.

IMMS, A. D., 1957. A General Textbook of Entomology. (9th Edition). Methuen, London.

ITO, T., 1953. Studies on the integument of the silkworm, *Bombyx mori*. VII. The permeability of the integument to oxygen and carbon dioxide *in vivo*. *Biol. Bull.*, **105**: 308–315.

ITO, T., 1954. Discontinuous output of carbon dioxide by undifferentiated *Bombyx* pupae. *Jap. J. Appl. Zool.*, **19**: 98.

LEVY, R. I., AND H. A. SCHNEIDERMAN, 1957. The direct measurement and significance of changes in intratracheal gas composition during the respiratory cycle of the Cecropia moth. *Anat. Rec.*, **128**: 583.

LEVY, R. I., AND H. A. SCHNEIDERMAN, 1958. An experimental solution to the paradox of discontinuous respiration in insects. *Nature*, **182**: 491–493.

MCCUTCHEON, F. H., 1940. The respiratory mechanism in the grasshopper. *Ann. Ent. Soc. America*, **33**: 35–55.

NUNOME, J., 1944. Kasan no Kotyu ni Kansuru Kenkyu. I. Sanji no Kokyu ni okeru Sanso no Kakusan ni Tsuite. (Studies on the respiration of the silkworm. I. Diffusion of oxygen in the respiratory system of the silkworm). (In Japanese). *Sanshi Shikenjo Hokoku (Bulletin of the Sericultural Expt. Station)* **12** (1) : 17–39. (Complete English translation from National Institutes of Health, courtesy Dr. John B. Buck).

PUNT, A., 1944. De gaswisseling van enkele bloedzuigende parasieten van warmbloedige dieren. (Cimex, Rhodnius, Triatoma). 141 *Onderzoekingen Physiol. Lab. Rijks. — Univ. Utrecht. 8th Ser. (Part III) : 122–141.*

PUNT, A., 1948. The respiration of insects during hibernation. *Acta Brevia Neerlandica*, **16**: 30.

PUNT, A., 1950a. The respiration of insects. *Physiol. Comparata et Occol.*, **2**: 59–74.

PUNT, A., 1950b. The influence of insecticides on respiration in insects. *Acta Physiol. et Pharmac. Neerlandica*, **1**: 82–89.

PUNT, A., 1956. Further investigations on the respiration of insects. *Physiol. Comparata et Occol.*, **4**: 121–131.

PUNT, A., W. J. PARSER AND J. KUCHLEIN, 1957. Oxygen uptake in insects with cyclic carbon dioxide release. *Biol. Bull.*, **112**: 108–117.

SCHNEIDERMAN, H. A., 1953. The discontinuous release of carbon dioxide by diapausing pupal insects. *Anat. Rec.*, **117**: 540.

SCHNEIDERMAN, H. A., 1956. Spiracular control of discontinuous respiration in insects. *Nature*, **177**: 1169–1171.

SCHNEIDERMAN, H. A., AND W. E. BECKEL, 1954. The coordination and control of spiracular valves in relation to discontinuous release of carbon dioxide by diapausing pupal insects. *Anat. Rec.*, **120**: 730.

SCHNEIDERMAN, H. A., AND C. M. WILLIAMS, 1953a. Physiology of insect diapause. VII. The respiratory metabolism of the Cecropia silkworm during diapause and development. *Biol. Bull.*, **105**: 320–334.

SCHNEIDERMAN, H. A., AND C. M. WILLIAMS, 1953b. Discontinuous carbon dioxide output by diapausing pupae of the giant silkworm, *Platysamia cecropia*. *Biol. Bull.*, **105**: 382.

SCHNEIDERMAN, H. A., AND C. M. WILLIAMS, 1955. An experimental analysis of the discontinuous respiration of the Cecropia silkworm. *Biol. Bull.*, **109**: 123–143.

Scholander, P. F., and F. J. W. Roughton, 1943. Microgasometric estimation of the blood gases. I. Oxygen. *J. Biol. Chem.*, **148**: 541–550.

Shimizu, S., and M. Ono, 1942. Kasan Yochu in okeru Kimon Heisakon no Undo ni tsuite (Movements of the closing apparatus of the stigma in the silkworm larva) (In Japanese). *Nihon Sanshigaku* (Japanese Journal of Sericulture), **13** (1) (Cited by Nunome, 1944: not seen in original).

Stahn, I., 1929. Über die Atmungsregulation, besonders die Kohlensäureregulation, bei *Dixippus morosus* und *Aeschna grandis*. *Zool Jahrb. allg.* **46**: 1–86.

Umbreit, W. H., R. H. Burris and J. P. Stauffer, 1958. Manometric Techniques and Tissue Metabolism. Burgess Publishing Co., Minneapolis, Minnesota.

Watts, D. T., 1951. Intratracheal pressure in insect respiration. *Ann. Entom. Soc. America*, **44**: 527–538.

Wigglesworth, V. B., 1935. The regulation of respiration in the flea, *Xenopsylla cheopis*, Roths. (Pulicidae). *Proc. Roy. Soc. London, Ser. B*, **118**: 397–419.

Wigglesworth, V. B., 1941. The effect of pyrethrum on the spiracular mechanism of insects. *Proc. Roy. Entom. Soc. (London), Ser. A*, **16**: 11–14.

Wigglesworth, V. B., 1953a. The Principles of Insect Physiology. Methuen, London.

Wigglesworth, V. B., 1953b. Surface forces in the tracheal system of insects. *Quart. J. Micr. Sci.*, **94**: 507–522.

Zeuthen, E., 1955. Comparative physiology (respiration). *Ann. Rev. Physiol.*, **17**: 459–482.